Risk Factors for Soil-Transmitted Helminth Infections in Schoolchildren from Rural Communities in Honduras

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Dedication

To my wife Ivonne and our children

María, José, Daniel and Gabriel

Abstract

Background: Honduras is endemic for soil-transmitted helminth (STH) infections. However, knowledge gaps remain in terms of risk factors involved in STH transmission and infection intensity.

Objectives: To determine the prevalence and intensity of STH infections in schoolchildren living in rural Honduras. Additionally, to investigate risk factors associated with STH infections.

Methods: A cross-sectional study was done among Honduran rural schoolchildren, in 2011. Demographic and epidemiological data were obtained and STH infections were determined using Kato-Katz method.

Results: A total of 320 children completed the study. Overall and specific prevalences for *Ascaris lumbricoides, Trichuris trichiura* and hookworms were 72.5%, 30%, 67% and 16%, respectively. Several risk factors associated with STH transmission and infection intensity were identified at the individual and familial level as well as at the schools.

Conclusions: Improving hygienic conditions and providing semi-annual deworming treatment are feasible interventions that could enhance undergoing STH control activities.

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List of abbreviations

CDC: Center for disease control

CI: Confidence interval

DALY: Disability adjusted life years

EPG: Eggs per gram (of stool)

FEC: Fecal egg counts

GEE: Generalized estimating equations

HIV/AIDS: Human immunodeficiency virus / Acquired immunodeficiency syndrome

LAC: Latin America and the Caribbean

MEIZ: Maestría en enfermedades infecciosas y zoonóticas (Masters program in infectious and zoonotic diseases)

NTD: Neglected tropical diseases

OR: Odd ratio

PAHO: Pan American Health Organization

SES: Socio-economic status

STH: Soil-transmitted helminth

UNA: Universidad Nacional de Agricultura (National University of Agriculture)

UNAH: Universidad Nacional Autónoma de Honduras (National Autonomous University of Honduras)

WHO: World Health Organization

CHAPTER 1: INTRODUCTION AND RESEARCH OBJECTIVES

Four intestinal helminths: *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), *Ancylostoma duodenale* and *Necator americanus* (hookworms) are collectively known as soil-transmitted helminths (STH) due to their ability to survive in the environment and be transmitted by fecally-contaminated soil (Bethony et al., 2006; WHO, 2002). Soil-transmitted helminth infections are among the most prevalent of chronic human infections worldwide and their distribution is closely related to climate and soil characteristics. Moreover, higher prevalences are found in tropical and sub-tropical regions, especially in developing countries where STH transmission is associated with poverty, lack of potable water, inadequate or inexistent sanitary facilities, poor hygiene and inefficient health services (Brooker et al., 2006; WHO, 2010, 2012).

According to recent estimates, more than 2 billion people worldwide are infected with STH (WHO, 2012), representing 40% of the global morbidity from infectious diseases (Hotez et al., 2003). Countries in Latin America and the Caribbean (LAC) account for 26% of the global number of STH infections. The size of infected population in LAC has been estimated as much as 100, 84 and 50 million for *Trichuris*, *Ascaris* and hookworms, respectively (de Silva et al., 2003; Hotez, 2011). In endemic communities, the majority of individuals usually harbour light infections while a few would have moderate to heavy infections (WHO, 2002). Polyparasitism is also a common finding in endemic areas. Morbidity is strongly related to intensity of infection, particularly in children, in whom STH can lead impairment of physical and mental development, which ultimately hinders educational progress and economic productivity. STH infections can also lead to absenteeism to school and adverse pregnancy outcomes (Guyatt, 2000; Hotez et al., 2008). STH distribution in endemic populations closely overlaps with those of poverty and malnutrition (Brooker et al., 2006; Hotez et al., 2008; Montresor et al., 2011; Weaver et al., 2010; WHO, 2012). Furthermore, STH transmission is intimately linked to environmental, climatic and cultural factors (Gazzinelli et al., 2012; Weaver et al., 2010).

STH infections are chronic and insidious, causing higher disability than mortality (Bethony et al., 2006); therefore, their burden of disease is better measured in DALYs (disability adjusted life years), the number of years lost due to ill-health, disability or early death (WHO, 2012). STH infections account for about 40 million DALYs globally, with the LAC region roughly accounting for 4 million DALYs (Hotez et al., 2008).

Honduras is one of the 30 countries in the LAC region endemic for STH (WHO, 2012). Although ranked as a medium-development country, almost 60% of Honduras population lives in poverty (earning less than two dollars per day); of these, about 40% live in extreme poverty (earning less than $1.25 per day). Moreover, of a total population of 8 million Hondurans, 2 million children live at risk of acquiring STH infections due to lack of sanitization, clean water and inadequate access to health care (WHO, 2012). According to national statistics, 13.7% (urban) and 32% (rural) of these children are undernourished (United Nations, 2010). According to the Pan American Health Organization (PAHO), gross estimates of the prevalence of intestinal worms in Honduras range from 12.2% to 97%, for a national average > 20% which is considered a generalized public health problem (PAHO, 2011). This is despite national efforts to control STH transmission by providing deworming treatment to large segments of the primary school population (WHO PCT databank, 2013). Notwithstanding STH high endemicity in Honduras, there is a paucity of research on the prevalence and intensity of infections and their impact on children's health. Moreover, epidemiological research to understand factors associated with transmission and infection intensity is virtually nonexistent in Honduras.

Therefore, studies examining STH risk factors could help elucidate particularities of transmission within the Honduras population. This knowledge could in turn inform control efforts so resources are maximized and effectiveness increased. To close this knowledge gap, the present study aims to identify STH infection risk factors in Honduran endemic communities and help further our understanding of STH transmission in the country.

1.1: Research goal

The main goal of the present study was to explore potential risk factors associated with STH transmission and infection intensity in primary school children residing in rural Honduras. To fulfill this goal, the following specific objectives were pursued:

1.1a: Specific research objectives

1. To determine the prevalence of STH infections in the study population.

2. To determine the intensities of STH infections in the study population.

3. To investigate whether or not demographic, socio-economic, behavioral and environmental conditions of the study population are associated with prevalence and intensity of STH infections.

CHAPTER 2: LITERATURE REVIEW

2.1: Soil-transmitted helminth (STH) infections

Soil-transmitted helminth (STH) infections are the most common of the neglected tropical diseases (NTD), a group of diseases strongly linked to poverty and deficient sanitary conditions that show endemicity in developing countries (Hotez, 2008a). The term STH refers to a group of nematode worms that are transmitted to humans by fecally-contaminated soil. *Ascaris lumbricoides, Trichuris trichiura, Necator americanus* and *Ancylostoma duodenale* are the species of major concern to humans (WHO, 2012).

2.1a: *Ascaris lumbricoides*

Ascaris lumbricoides (Linnaeus, 1758), the common roundworm, is the largest intestinal parasitic nematode causing infections in humans. This parasite is a member of the family Ascarididae, and closely related to the swine parasite *Ascaris suum* (Roberts and Janovy, 2009a). Adult worms usually reside in the jejunum but can be found in the entire small intestine especially when they are present in large numbers (Bethony et al., 2006). These worms live freely in the intestinal lumen, not attached to the mucosa, and absorb nutrients directly from the hosts' intestinal content through their buccal cavity which is oriented to the intestinal flow (Anderson, 1992; Hotez et al., 2003). The adult worm's lifespan is about a year, sometimes up to 20 months and upon dying, they are expelled with the feces. Sometimes worms may migrate into unusual sites such as bile and pancreatic ducts causing obstruction and life-threatening complications (Beaver et al., 1992; Hall et al., 2008).

Female *Ascaris* are larger (40 cm) and heavier (up to 9 g) than males which can weigh up to 2 - 3 g and grow as long as 30 cm. Males have a curled posterior end while females have a straight one (Figure 1). Approximately 9-11 weeks post infection, mature females start laying between 100,000 – 200,000 fertilized or unfertilized (those produced in the absence of mating) eggs per day (Bethony et al., 2006; Hotez et al., 2003; Sinniah, 1982). There is not a linear relationship between the number of worms in a host and the amount of eggs shed in feces since the latter depends on several variables such as daily egg output, worm load and the age of the worms (Sinniah, 1982). In addition, there is evidence that fecundity varies based on the geographical zone and the worms' population density in the host (Hall and Holland, 2000).

A. lumbricoides eggs are passed out in host's feces and thus finding them in stool samples are the basis for the diagnosis infection. These eggs are depicted in Figure 1.Typically, fertilized eggs are 45 - 75 µm length, round-shaped and have a thick shell with an external mammillated (rough, bumpy) layer often stained brown by bile. However, sometimes the outer layer can be absent (known as decorticated eggs). Unfertilized eggs are easy to identify since they are elongated and larger than fertilized eggs (up to 90 µm in length), contain refractile granules, have a thinner shell, and their mammillated layer can either show large protuberances or be practically non-existent. Unfertilized eggs contain mainly a mass of refractile granules (Anderson, 1992; Bogitsh et al., 2012).

Recently excreted eggs in feces are not infective and require a period of maturation in the soil to embryonate. After this period, a larva is produced within the egg, which then molt into a second-stage larva (L2), the infective stage to humans. This maturation period varies according to environmental conditions, primarily temperature, and may range from two weeks to several months (Beaver et al., 1992; Hall et al., 2008; Hotez et al., 2003). Shorter maturation period takes place at 25 - 30 ºC while longer occurs at around 17 ºC. *Ascaris* eggs are resistant to desiccation and may remain viable in the soil for up to two years or more (Anderson, 1992; Beaver et al., 1992). Despite their resistance, eggs in the embryonic stage are still susceptible to environmental temperature: maturation process ceases below 15.5 °C and viability is seriously compromised above 38 °C (Bethony et al., 2006; Hall et al., 2008).

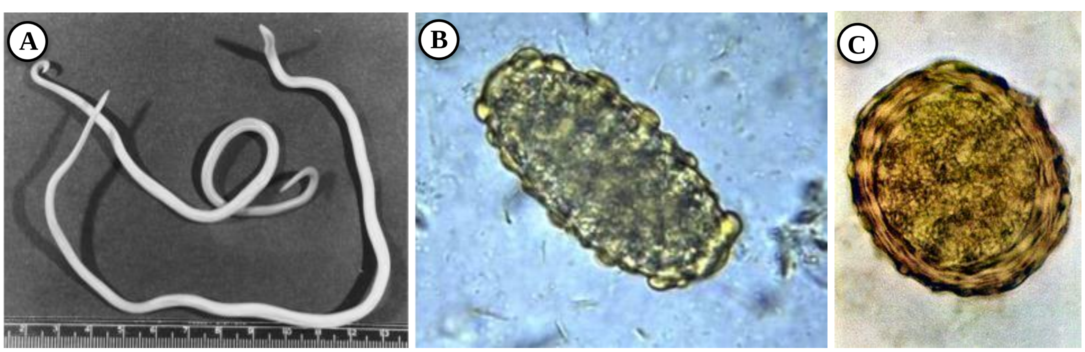


Figure 1. *Ascaris lumbricoides*. (A) Adult specimens, curled end in males is observable; (B) unfertilized egg with refractile granules; (C) fertilized egg with typical external mammillated layer (CDC, 2012)

The life cycle of *A. lumbricoides* is illustrated in Figure 2. Following the ingestion of infective eggs and when in contact with bile, L2 larvae hatch in small intestine where they penetrate the mucosa and migrate to the lungs using the portal and systemic circulation. Once in the lungs, larvae grow and undergo two additional molts becoming fourth stage larvae (L4). At this point, L4 larvae penetrate the alveolar space, ascend to the pharynx, are coughed up and then swallowed, returning this way to the small intestine. Once in the intestine, L4 larvae grow and undergo their last molt developing into adult worms. It takes between 2 - 3 months from the ingestion of the infective eggs to oviposition by mature female worms (Bethony et al., 2006; Bogitsh et al., 2012; Dold and Holland, 2011; Hotez et al., 2003).

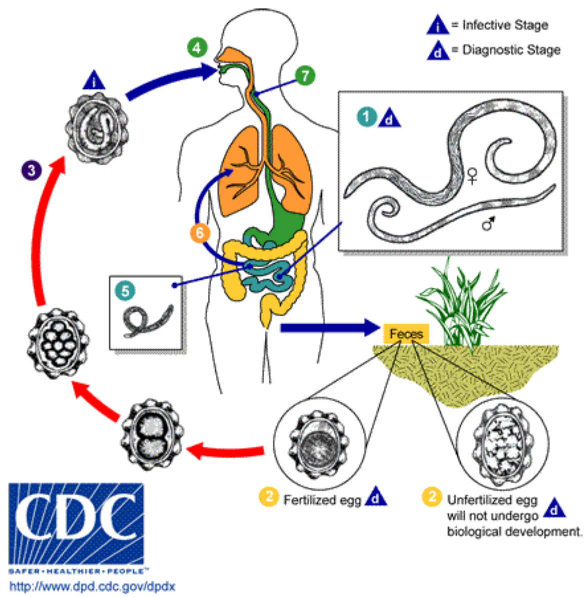


Figure 2. Life cycle of *Ascaris lumbricoides* (CDC, 2012)

2.1b: ***Trichuris trichiura***

Trichuris trichiura (Röderer, 1761) belongs to the nematode superfamily Trichuroidea; it is commonly called human whipworm due the whip-like shape of the adult. Adult worms reside in the large intestine, especially in cecum. However, in heavy infections, parasites can spread throughout the colon and even reach the rectum (Hotez et al., 2003). Contrary to *Ascaris*, *Trichuris* does not live free in the intestinal lumen but lives attached to the intestinal mucosa. Helped by secreting pore-forming proteins to perforate the epithelial tissue, it embeds its thin anterior portion into the intestinal epithelium where it matures and feed on tissue secretion but not blood. The posterior end of the worm protrudes freely into the lumen (Bethony et al., 2006; Bogitsh et al., 2012; Hotez et al., 2003). Generally, their lifespan is about 1.5 - 2 years (Bethony et al., 2006), however exceptionally longer infections of 8 years or more have also been reported (Bogitsh et al., 2012; Shiff, 2007).

As mentioned above and depicted in Figure 3, adult worms have a whip-like shape with a thin anterior portion and a markedly wider posterior portion (Bogitsh et al., 2012). Males can grow as long as 3 - 5 cm; they are slightly smaller than females and can be easily distinguished by their coiled posterior end (Bethony et al., 2006). Approximately 60-70 days post infection fertilized females start laying between 3,000 – 20,000 eggs per day (Bundy and Cooper, 1989; Hall et al., 2008). As illustrated in Figure 3, eggs are typically barrel-shaped with two polar plugs (called opercula). As in the case of *Ascaris*, eggs are expelled to the environment in the host's feces and constitute the diagnostic stage. Recently excreted eggs require a period of maturation in soil (2 - 4 weeks at 22 ºC in humid soil) in order to embryonate and produce a first-stage larva (L1), the infective stage to humans (Bogitsh et al., 2012; Hotez et al., 2003; Nejsum et al., 2012).

Similar to *Ascaris*, *Trichuris*' maturation period is affected by temperature fluctuations and larval development can take up to several months if temperature fluctuates between 6 - 24 ºC. *T. trichiura* eggs perish below -9 ºC or above 52 ºC. They can survive and remain infective for several months, and even years, in warm moist soil, but cannot survive under dry conditions or direct sunlight exposure (Cheesbrough, 1992; Stephenson et al., 2000).

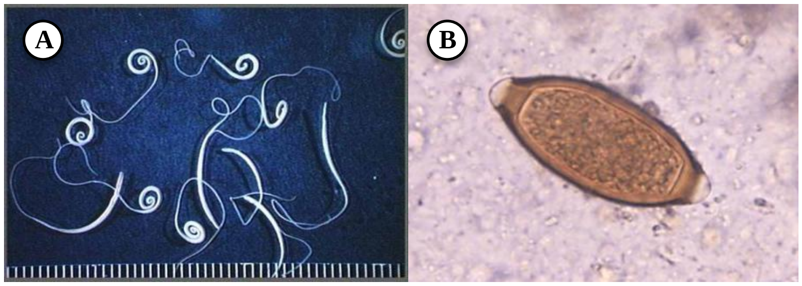


Figure 3. *Trichuris trichiura*. (A) Adult specimens, coiled end in males is evident; (B) barrel-shaped egg with polar opercula (CDC, 2012)

The life cycle of *T. trichiura* is illustrated in Figure 4. Following the ingestion of infective eggs and when in contact with bile, L1 larvae hatch in small intestine and penetrate into the crypts of Lieberkühn where they grow and undergo four molts until developing into adults. Juvenile adults migrate to cecum and penetrate the mucosa feeding on tissue secretions and reaching sexual maturity. It takes between 2 - 3 months from the ingestion of the infective eggs to oviposition by mature fertilized female worms and the ensuing appearance of diagnostic eggs in the stools (Bogitsh et al., 2012; Hotez et al., 2003; Nejsum et al., 2012; Stephenson et al., 2000). From this description, it can be seen that *T. trichiura* does not require heart-lung migration as *Ascaris* does.

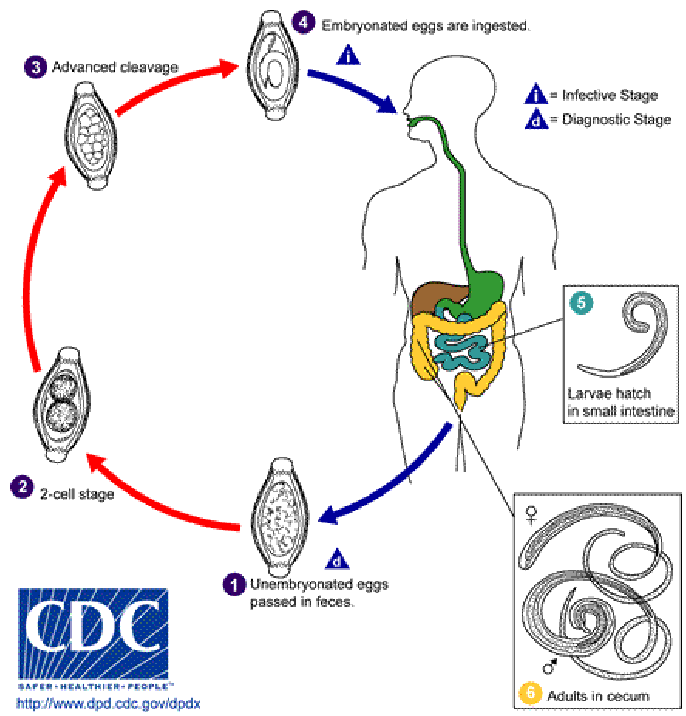


Figure 4. Life cycle of *Trichuris trichiura* (CDC, 2012)

2.1c: Hookworms

In humans, the term hookworms describes two species of parasitic nematodes, *Necator americanus* (Creplin, 1845) and *Ancylostoma duodenale* (Stiles, 1903), both belonging to the nematode superfamily Strongyloidea. They are grouped together because both occur worldwide and have an overlapping geographical distribution; moreover, the eggs of each species are identical and cannot be differentiated when examined under a microscope (Hall et al., 2008). *N. americanus* parasitizes mainly humans although found in dogs while *A. duodenale* infections have also been found in felines and canines (Hotez, 2008a). Adult worms parasitize the upper part of small intestine, however in severe infections they can be found as low as the ileum (Gilman, 1982). They live attached to the intestinal mucosa using specialized oral cutting organs. Both species feed from host's blood and tissue fluids (Hall et al., 2008; Hotez et al., 2003). *Ancylostoma duodenale* is considered more pathogenic because it produces greater blood loss than *Necator americanus* (about 0.2 mL per day and 0.04 mL per day, respectively) (Bogitsh et al., 2012; Hall et al., 2008; Hotez, 2008a). The lifespan for *A. duodenale* is 5 - 7 years while *N. americanus* can live up to 20 years, although the majority of the population dies in 1 - 2 years (Bethony et al., 2006; Bogitsh et al., 2012; Gilman, 1982).

Adult worms of both species are very similar, measuring up to 0.8 - 1.5 cm in length (Figure 5A). Males are slightly smaller than females and have an umbrella-shaped copulatory bursa in their posterior end (Bethony et al., 2006; Bogitsh et al., 2012). As showed in Figure 5B, adults of both species can be differentiated by their oral cavity, which contains teeth in *A. duodenale* or cutting plates in *N. americanus*. Females of *A. duodenale* have greater fecundity, laying approximately 10,000 – 25,000 eggs per day, while females of *N. americanus* lay 5,000 – 10,000 eggs per day (Bethony et al., 2006; Hotez et al., 2003; Pawlowski et al., 1991). Similar to *Ascaris* and *Trichuris*, hookworm eggs are expelled in host's feces and constitute the diagnostic stage. Eggs of both species are identical (Figure 5C): 64 - 76 x 36 - 40 µm, bluntly rounded ends, transparent shell and, once in feces, embryonated with 4 - 8 cell morulae (Bogitsh et al., 2012).

Similar to *Ascaris* and *Trichuris*, hookworms' maturation period depends on temperature and humidity. However, hookworms' optimal environment is sandy, shaded soil rich in organic material, and a temperature between 23 - 33 ºC, where eggs can embryonate to L1 larvae in 1 - 2 days and hatch from eggs living freely in the soil. After growing and molting twice, L1 larva becomes a non-feeding but motile L3 larva, the infective stage (Bethony et al., 2006; Bogitsh et al., 2012). L3 larvae's lifespan is only few weeks and their survival depends on environmental conditions, being longer in places with sandy soils, warm temperatures and relatively high rainfall (Mabaso et al., 2003).

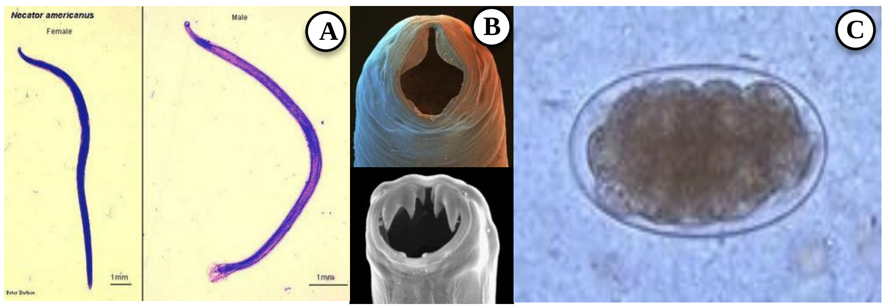


Figure 5. Hookworms. (A) *N. americanus* adult specimens, male exhibit a copulatory bursa in posterior end; (B) oral cavity of *N. americanus* (top) and *A. duodenale* (bottom); (C) morulated egg (CDC, 2012)

The life cycle of hookworms is shown in Figure 6. L3 larvae remain close to the area where feces were deposited, inhabiting the upper 10 cm of soil to improve chances of contact with human skin (Bethony et al., 2006; Bogitsh et al., 2012; Gilman, 1982; Hall et al., 2008). Infection with both species of hookworm occurs when L3 larvae penetrate bare skin (usually feet and legs). After penetration, L3 larvae are carried through the portal circulation to the heart and then the lung, where they break into the alveoli and migrate up the respiratory tree, molting en route (becoming L4 larvae). From the respiratory tree, larvae are coughed up and swallowed reaching the small intestine, where they molt once more and develop into mature adults. The heart-lung migration can last approximately one week. It will take about 4 - 6 weeks post-penetration for becoming sexually mature adults and start laying eggs (Bethony et al., 2006; Bogitsh et al., 2012; Hall et al., 2008; Nelson and Masters, 2007). Adults feed by sucking part of the intestinal mucosa into their oral cavity and cutting it using their mouthparts. Worms change attachment site frequently and abandoned sites may keep bleeding for some time (Gilman, 1982; Watson and Hickey, 2010). Unlike *N. americanus*, L3 larvae of *A. duodenale* can also infect via the oral route. Additionally, lactogenic transmission during breastfeeding has been postulated although evidence is not conclusive (Bethony et al., 2006; Bogitsh et al., 2012; Schad et al., 1973; Tiwari et al., 2004; Yu et al., 1995). In Honduras, where *N. americanus* is the prevalent species, the first reported case of *A. duodenale* occurred in a two-month infant who had been exclusively breastfed. Lactogenic transmission was suspected (Kaminsky, 2000).

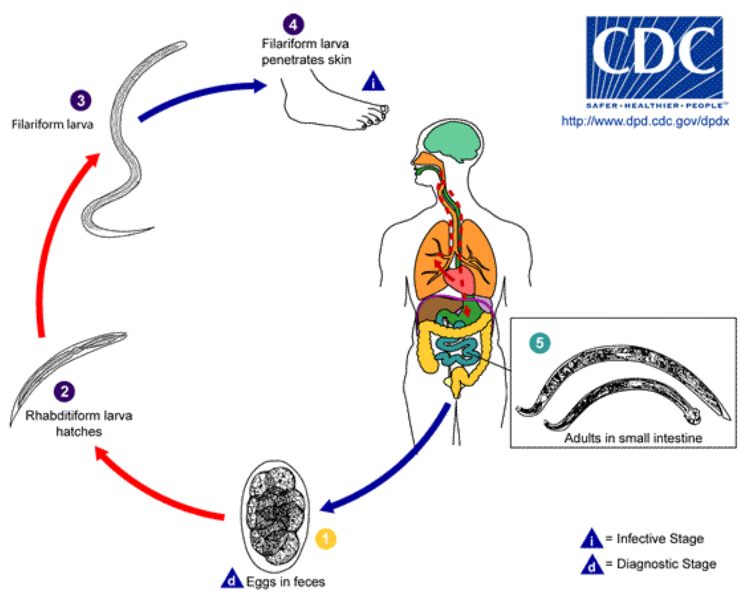


Figure 6. Life cycle of Hookworms (CDC, 2012)

2.2: Health impact of soil-transmitted helminth infections

As mentioned before, STH’s life cycles are complex and both larvae and adults can cause disease in the host. Larval stages are associated with acute disease while adults are associated to both chronic and acute disease (Murray et al., 2004).

2.2a: Larval migration

Both *Ascaris* and hookworms undergo larval migration in order to complete their life cycles. During this migration, the host can suffer acute pathology as described below.

Ascaris larvae can cause eosinophilic pneumonia (previously known as ‘Löeffler syndrome’), an intense inflammatory response in lungs characterized for heavy eosinophilic infiltrate, wheezing, dyspnea, non-productive cough, fever and, in heavy infections, bloody sputum (Aggarwal et al., 2008; Hoenigl et al., 2010; Hotez et al., 2003). Occasionally, eosinophilic granuloma and liver damage have also been reported due larval migration of *Ascaris suum* (Hayashi et al., 1999).

Upon skin penetration, hookworm larvae can cause a variety of cutaneous manifestations among which the most common is a mild allergic reaction (‘ground itch’) characterized by local erythematous, pruritic and papular rash usually on the hands and feet. In contrast, zoonotic hookworm infections develop into *cutaneous larva migrans*, a self-limiting dermatological condition characterized by serpiginous tracks (1 - 5 cm long) on the feet, buttocks or abdomen. During the heart-lung migration, hookworm larvae can also cause pneumonitis but allegedly milder than the observed in *Ascaris*. Oral ingestion of *A. duodenale* larvae, especially in large amounts, can result in Wakana Syndrome, a process similar to an immediate-type hypersensitivity reaction characterized by nausea, vomiting, pharyngeal irritation, cough, dyspnea, and hoarseness (Bethony et al., 2006; Gilman, 1982; Hall et al., 2008; Hotez et al., 2004; Hotez et al., 2003; Schuster et al., 2011).

2.2b: Adult stage

Since the final habitat of STH adult worms is the human intestine, functional and physiological intestinal alterations can occur (Table 1). These alterations can lead to either acute or chronic manifestations, some of the latter due to continuous nutrient depletion. In children, nutrients loss can lead to stunting, reduced physical activity and decreased learning ability (Montresor et al., 2011). Usually morbidity is associated with worm burden (i.e., number of adult worm living in the intestine); the more severe conditions can be seen in higher intensities (Albonico et al., 2008). However, morbidity is not only associated to intensity of infection but with chronicity of infection as well. Indeed, low intensity infections have been also associated with a significant health detriment, especially in children (Crompton and Nesheim, 2002; Stephenson et al., 2000). Additionally, the health impact of concomitant infections has been study and empirical results suggested that, especially in children, low-intensity polyparasitism has an additive or synergistic detrimental effect (Ezeamama et al., 2005; Pullan and Brooker, 2008; Steinmann et al., 2010).

Table 1. Conditions associated with STH infections (adapted from Montresor et al., 2011)

Type of condition	Signs of condition	Parasite
Nutritional impairment	Intestinal bleeding, anemia	Hookworms
	Malabsorption of nutrients	*A. lumbricoides*
	Competition for micronutrients	*A. lumbricoides*
	Impaired growth	*A. lumbricoides*
	Loss of appetite, reduced food intake	*A. lumbricoides*
	Diarrhea or dysentery	*T. trichiura*
Cognitive impairment	Reduced verbal fluency and memory	*T. trichiura* *A. lumbricoides* Hookworms
Conditions requiring surgical intervention	Intestinal / biliary obstructions	*A. lumbricoides*
	Rectal prolapse	*T. trichiura*

***Ascaris lumbricoides*.** Approximately 85% of ascariasis cases are asymptomatic, however non-specific upper abdominal pain, nausea, anorexia, diarrhea, vomiting and weight loss have been found associated with this infection. Adult worms can lead to malnutrition and stunting not only due to nutrients taken from the host but also by the protein digestion impairment produced, with the consequently loss of appetite and malabsorption of fat, lactose and vitamin A in the host (Bogitsh et al., 2012; Hotez, 2008b). Large number of worms can result in worm masses that may eventually obstruct the intestinal tract and require surgical intervention. In addition, adult worms can penetrate the intestinal wall causing local hemorrhage, peritonitis, and/or appendicitis (Hotez et al., 2003; Valentine et al., 2001). Migration of adult worms can cause biliary and pancreatic obstruction, conditions that are more common in adults than children (Bogitsh et al., 2012; Grover et al., 2001). These acute complications can be fatal. In

Honduras, the first report of biliary ascariasis was done by Lozano (1955). Since then, a total of 39 cases of biliary ascariasis have been reported (90% in adults) (Fajardo et al., 2003; Galeas-Castillo and Durón, 1998; Lozano, 1955; Murillo and González, 2011; Núñez, 1990; Zavala and Ruiz, 1989; Zúñiga et al., 1960).

***Trichuris trichiura*.** Adult worms live embedded in the colon's mucosa and, if in large amounts, can cause eosinophilic colitis, an inflammatory process clinically characterized by abdominal pain and diarrhea (Baldisserotto, 2010; Okpara et al., 2009). Many light infections appear to be asymptomatic but when severe symptoms arise, they include chronic bloody diarrhea with abundant mucus, weight loss, severe stunting, severe anemia and rectal prolapse in young children (Albonico et al., 2008; Stephenson et al., 2000). This clinical presentation, named as *Trichuris* dysentery syndrome (TDS), is the most severe manifestation of trichuriasis and usually occurs in children although cases in adults have been reported (Hotez et al., 2003; Khuroo and Khuroo, 2010). Fatalities can result when infections are massive (Cheesbrough, 1992). In Honduras, only one publication was found associating pathologies to heavy *Trichuris* infections. Four children (5 - 7 years old) were diagnosed with dysentery, moderate to severe anemia (3.5 - 9.4 g/dL) and acute malnutrition. One child presented cognitive impairment but no fatalities were reported (Kaminsky et al., 2010).

Hookworms. Since adult worms are hematophagous, the most important pathology associated with these infections is intestinal blood loss, which may lead to iron-deficiency anemia. It is estimated that as few as 40 adult worms can drop haemoglobin levels to values lower than 11 g/dL, although it appears that blood loss can be lower in *N. americanus* infections (Bethony et al., 2006; Hotez et al., 2003). Because children and women in reproductive age have reduced iron reserves, they are at particular risk of developing anemia. In pregnant women, severe anemia can result in life-threatening condition for the mother, the fetus and the neonate (Bethony et al., 2006; Brentlinger et al., 2003; Brooker et al., 2008; Gyorkos et al., 2011a). Other important clinical manifestations of these infections are hypoproteinaemia and anasarca – extreme generalized edema – (Bethony et al., 2006; Hotez et al., 2004), stunted growth and delayed puberty (Bogitsh et al., 2012), mental dullness and cognitive impairment in children (Bogitsh et al., 2012; Jardim-Botelho et al., 2008; Sakti et al., 1999). In Honduras, heavy hookworm infections have been associated to severe anemia and edema in extremities in one adult (Cáceres, 1934), massive gastrointestinal bleeding in two infants (Dala et al., 1991) and anemia and eosinophilia in a two-months old infant (Kaminsky, 2000).

2.3: Diagnosis of soil-transmitted helminth infections

Diagnosis of STH infections based on clinical symptoms can be misleading, since similar symptoms can be observed in several pathologies. Therefore, STH infections are typically diagnosed by identifying the parasites' eggs (diagnostic stage) in a microscopic examination of stool specimens. With the proper training, species can be easily identified based on their eggs' morphological features (except between hookworm species since both have identical eggs) (Bogitsh et al., 2012; WHO, 1991). Occasionally adult worms can be passed in feces (especially after deworming treatment), in which case identification can also be made based on morphological characteristics of the adult worms. When rectal prolapse occurs, adult worms of *T. trichiura* can be observed externally, embedded in the rectal mucosa (Bogitsh et al., 2012).

Different laboratory techniques can be used to detect STH diagnostic stages in stool samples, from the direct wet mount to a variety of concentration techniques. In light infections, which tend to be the majority, the direct wet mount, which is rather insensitive, can fail to detect the infection. Therefore, concentration techniques should be employed in both clinical routine diagnosis and epidemiological studies for increasing the probability of finding the parasites (Bogitsh et al., 2012; WHO, 1994).

Direct wet mount. It is the simplest and easiest technique for examination of feces (WHO, 1991). Approximately 2 mg of feces is suspended in either isotonic saline or 1% Lugol's iodine solution. It is employed primarily to demonstrate helminth eggs, larvae, protozoan trophozoites, and cysts. A good identification can be done since this method preserve helminth eggs' characteristic features. In 1950, a standardized method for egg counting in direct wet mounts was introduced by Beaver for estimating worm load of *Ascaris* and *Trichuris* (Beaver, 1950). Nevertheless, an important disadvantage is that direct wet mount has very low sensitivity and therefore should not be used alone for routine diagnosis or epidemiological studies (Neimeister et al., 1990).

Concentration methods. Concentration methods can be based on sedimentation or flotation techniques, the latter are the most frequently used for diagnosing STH infections (Knopp et al., 2011a; Mahon et al., 2007). Based on sedimentation principle, formol-ether (and its variants) is a widely used technique for concentrating cysts, eggs and larvae. It is very useful for heavy trematodes' eggs; however, in case of *Taenia* and *Ascaris* eggs –

especially infertile *Ascaris* – the concentration can be unsatisfactory (Allen and Ridley, 1970).

Flotation techniques are based on a density differential between helminth's eggs and a dense flotation solution (specific gravity of about 1.20), allowing the eggs to float to the top from where they can be skimmed onto a microscope slide. Zinc sulphate, magnesium sulphate, sodium nitrate and sucrose are commonly used as flotation solutions (Mahon et al., 2007).

In addition to STH determination for diagnostic purposes, the estimation of the infection intensity becomes essential for planning and/or following-up any possible intervention toward STH control in a particular population, especially for evaluating anthelmintic efficacy. Quantitative techniques such as McMaster, FLOTAC and Kato-Katz allow calculating the number of eggs per gram (epg) of stool specimen and, are very useful for estimating the intensity of infection (Montresor et al., 1998; WHO, 2002).

McMaster egg counting technique. Widely used in veterinary studies, this technique combines a flotation method with a counting chamber that allows the microscopical examination of a known volume (0.30 mL) of fecal suspension. Considering the amount of sample examined (20 mg), the number of eggs counted for each helminth species is then multiplied by a factor of 50 to obtain the number of eggs per gram (epg) in the sample (Mahon et al., 2007; Vercruysse et al., 2011b). It has been proposed as an alternative method for monitoring large-scale treatment programs (Levecke et al., 2011).

FLOTAC technique. This is a recently developed flotation technique that has been gaining wide acceptance and usage in both human and veterinary studies (Becker et al., 2011). Advocates for this technique claim it has better sensitivity than McMaster and Kato-Katz techniques especially in light infections and, particularly in hookworm infections (Cringoli et al., 2010; Glinz et al., 2010; Knopp et al., 2009; Utzinger et al., 2008). However, FLOTAC technique is quite complex and requires the use of special instruments (Cringoli et al., 2010), cost about 35% more than Kato-Katz (Speich et al., 2010) and yields lower fecal egg counts (FEC) than Kato-Katz (Habtamu et al., 2011). These are limitations to be considered before using it in large-scale epidemiological studies.

Kato-Katz technique. 'Kato' thick smear technique for helminth eggs quantification was firstly introduced in 1954 by Kato and Miura (Kato and Miura, 1954). Later in 1972 the name of this technique changed to 'Kato-Katz' after the modification done by Katz et al.: to overcome the need of weighing fecal specimens, they introduced a fecal measuring device (a disposable cardboard template with a hole) allowing to use a similar amount of sample described in the original method (45 mg of fecal material), (Katz et al., 1972). This modification made the technique more reliable, cheaper and easy to perform. These attributes launched Kato-Katz technique's popularity for surveillance and epidemiological surveys. This technique has been widely diffused by WHO/PAHO for qualitative and quantitative diagnosis of STH infections and is preferred over the direct wet mount (Montresor et al., 1998; WHO, 1994, 2002). The rationale for this recommendation from WHO are the following:

a. Kato-Katz uses more sample than the direct wet mount (41.7 mg vs. 2 mg, respectively), increasing diagnostic sensitivity (WHO, 2002).
b. It is easy to perform, relatively cheap, accurate and suitable for use in large-scale settings (Glinz et al., 2010; Tarafder et al., 2010; WHO, 2002).
c. Helminth eggs are highly visible due the clearing process of fecal material, improving microscopic observation and identification (Santos et al., 2005).
d. The standardized template used for the procedure delivers approximately 41.7 mg of fecal material so the number of eggs in the sample can be calculated. The number of eggs of every STH species counted in the Kato-Katz smear is then multiplied by a factor of 24 for obtaining the number of egg per gram (epg) and estimating the intensity of infection (Knopp et al., 2008; Montresor et al., 2011; WHO, 2002). WHO's classification of infection intensity is shown in Table 2.

Despite Kato-Katz widespread acceptance, some authors have brought attention to the fact that performing the method as recommended by WHO, leads to low sensitivity in case of light infections, particularly after deworming. They suggest increasing sensitivity by either preparing several smears from the same stool sample or by collecting several samples in consecutive days (Becker et al., 2011; Glinz et al., 2010; Knopp et al., 2011a). Tarafder and co-workers (2010), however, in their Bayesian analysis of data obtained from 5,624 individuals participating in a longitudinal study in Philippines, demonstrated that Kato-Katz sensitivity in one stool sample was adequate: 96.9%, 91.4% and 65.2%, for *A. lumbricoides*, *T. trichiura* and hookworms, respectively. In addition, specificity estimates for one stool sample were 96.1%, 94.4%, and 93.8%, for *A. lumbricoides*, *T. trichiura* and hookworm, respectively. The low sensitivity found for detection of hookworm infections may be related to rapid degeneration of delicate hookworm eggs with time (Tarafder et al., 2010). The latter has also been studied by Dacombe and colleagues (2007) who showed that the sensitivity of hookworm detection in stool specimens decreases with time. Approximately a 50% decrease in sensitivity for detection hookworms was observed when samples' refrigeration was delayed by more than 3 hours or when laboratory processing (using refrigerated samples) was delayed by more than one day (Dacombe et al., 2007). Additionally, Kato-Katz clearing time is critical since hookworm eggs can be easily destroyed by the glycerol used in the process ('overclearing') resulting in underestimation of prevalence (Knopp et al., 2008; WHO, 1994). For avoiding the overclearing slides should be examined promptly, within 30 - 60 minutes (Santos et al., 2005; WHO, 1994) or 30 - 90 minutes (Booth et al., 2003) after preparation.

2.3a: Estimation of intensity of STH infections and worm load

According to WHO, the intensity of STH infections can be measured directly, by counting the number of expelled worms after deworming, or indirectly estimated by fecal egg count (FEC), that is, counting the numbers of helminth eggs excreted in feces (expressed as epg). In 1987, a WHO Expert Committee recognized that using aggregated data and a reasonable sample of subjects ('reasonable sample' was not defined) egg mean concentration in feces is generally representative of the worm load. As a result, thresholds in egg concentration were set to define 'light', 'moderate' or 'heavy' infections of each of the three STH species (Montresor et al., 1998; WHO, 1987, 2002). Table 2 shows these categories, based on the epg count in a stool specimen. This method for categorizing STH infections is quite accepted although some authors consider this scientifically dubious (Hall et al., 2008; Hall and Holland, 2000)

Table 2. Fecal egg count thresholds of intensity of infections in soil-transmitted helminths (WHO, 2002)

STH species	**Threshold of intensity of infection (epg)**		
	Light	**Moderate**	**Heavy**
Ascaris lumbricoides	1-4,999	5,000-49,999	≥ 50,000
Trichuris trichiura	1-999	1,000-9,999	≥ 10,000
Hookworms	1-1,999	2,000-3,999	≥ 4,000

*epg: eggs per gram of feces

As mentioned previously, even when host-related factors must play an important role, STH morbidity is primarily determined by the worm load (number of adult worms in the host), this is, the infection intensity (Crompton and Nesheim, 2002; Pawlowski, 1987; Stephenson et al., 2000). That is why global public health efforts are essentially oriented to decrease morbidity through reducing intensity of infection in high-risk populations. In this context, the accuracy in estimating STH infection intensity and prevalence becomes crucial for decision-making and for monitoring control programs (Montresor et al., 2011; WHO, 2012).

Therefore, the indirect estimation of infection intensity through FEC becomes the cornerstone of control strategies. Fortunately, worm loads are fairly stable over short periods of time in a given population. This is because STH do not complete their life cycle within the host, so any changes in worm load is due to either loss of adult worms or gain of infective stages that later become adults. Nevertheless, FEC involves many assumptions as well as potential sources of inaccuracy (Hall, 1982; Hall and Holland, 2000). Sources of FEC variability include factors related to the parasite as well as to factors intrinsic to the host. Some of these factors are discussed below.

Parasite-related factors for FEC variability:

a) Only females produce eggs after fertilization by males (except for *Ascaris* that is able to lay infertile eggs). Sometimes even number of males is assumed (Hall, 1982) while some studies of *A. lumbricoides* have estimated a male:female ratio of 1:1.4 (Sinniah, 1982; Sinniah and Subramaniam, 1991). On the other hand, infection with unfertilized *Ascaris* (females alone) are considered to be common (Stoll, 1951). Thus, assuming any kind of ratio can lead to an inaccurate estimation of the worm load.

b) During pre-patency, eggs are not produced at all or produced in such small amount to be detected and false negatives can be diagnosed (Bogitsh et al., 2012; Hall, 1982).

c) Oviposition can be delayed until environmental conditions are favourable. This phenomenon known as 'arrested development' has been described for hookworm infections (Hotez et al., 1993; Schad et al., 1973).

d) Eggs production may be reduced in parasites' crowding conditions (Anderson and Schad, 1985; Bundy and Cooper, 1989; Krupp, 1961; Sinniah and Subramaniam, 1991; Walker et al., 2009).

e) Eggs production may be related to worm age after the pre-patent period. Eggs production peak is observed when maturity is reached but it will eventually decrease with worm aging (Hoagland and Schad, 1978; Sinniah and Subramaniam, 1991).

<u>Host-related factors for FEC variability</u>:

a) Eggs are diluted by the amount of feces produced. Fecal output varies in every individual according to the amount of food eaten, water and fibre content, and whether diarrheal disease is present. An increased fibre intake can sharply increase (50 - 120%) the amount of feces produced daily (Cummings and Stephen, 1980).

b) The size of the host normally determines the amount of food eaten, with the abovementioned implications in feces production. In general, children produce less amount of feces than adults (Hall, 1982).

c) Eggs may be unevenly mixed in feces. Considering the peristaltic movement of the intestine and transit time of feces, a more even distribution of eggs in feces would be expected for those STH living in the small intestine (*i.e.*, *Ascaris* and hookworms) than in *Trichuris*, residing in the large intestine. However, very few studies have examined this and results are still contradictory (Hall, 1981; Krauth et al., 2012; Ye et al., 1997).

d) The host's immunological response can affect fertility and fecundity of worms, acting in a density-dependant manner (Hall, 1982).

2.4: Geographic distribution and prevalence of STH infections

Soil-transmitted helminth infections are among the most prevalent of chronic human infections worldwide. STH distribution in a country is closely related to climate and soil characteristics. The major endemic regions include south and south-west China, southern regions of India, south-east Asia, sub-Saharan Africa, and Central and South America as shown in Figure 7 and Table 3 (de Silva et al., 2003; Savioli and Albonico, 2004). Higher prevalences are found in tropical and sub-tropical regions, especially in developing countries where STH infections are associated to poverty, lack of potable water, inadequate or non-existent sanitary facilities, poor hygiene and inefficient health services (Brooker et al., 2006; WHO, 2010, 2012). For the purpose of this thesis, emphasis was made on STH prevalence in Latin America and the Caribbean and more specifically in Honduras.

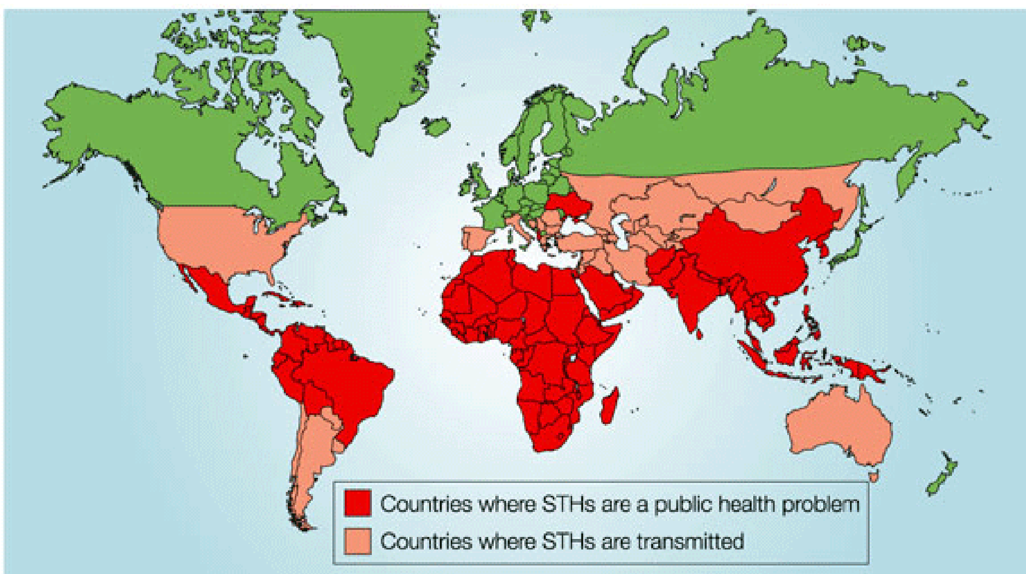


Figure 7. Global distribution of soil-transmitted helminth infections (Savioli & Albonico, 2004)

Table 3. Global estimate of prevalence of soil-transmitted helminth infections by regions (adapted from de Silva et al., 2003)

STH species	Infection prevalence (%)[a]							
	LAC	SSA	MENA	SAS	India	EAP	China	Total
A. lumbricoides	16	25	7	27	14	36	39	26
T. trichiura	19	24	2	20	7	28	17	17
Hookworms	10	29	3	16	7	26	16	15

LAC: Latin America and the Caribbean; SSA: Sub-Saharan Africa; MENA: Middle East and North Africa; SAS: South Asia; EAP: East Asia and the Pacific Islands
[a] Based on the estimated population in 2002

2.4a: Latin America and the Caribbean (LAC)

Roughly, the size of the infected population in LAC countries has been estimated to be as much as 100, 84 and 50 million for *Trichuris*, *Ascaris* and hookworm, respectively; accounting for 26% of the global burden of STH infections (Table 4). These figures are not surprising considering that in the LAC region about 195 million people live in poverty (earning less than 2 dollars per day), 71 million of them living in extreme poverty (earning less than $ 1.25 per day). Poverty conditions make people more susceptible to infectious diseases, including intestinal parasitism (Schneider et al., 2011).

Table 4. Global burden of soil-transmitted helminthiasis (adapted from de Silva et al., 2003 and Hotez, 2011)

STH species	Nº of infections worldwide (million)	Percent Global Disease Burden in LAC	Global disease burden in DALYs (million)	Deaths
A. lumbricoides	1,221	6.9%	1.2 – 10.5	3,000 – 60,000
T. trichiura	795	12.6%	1.6 – 6.4	3,000 – 10,000
Hookworms	740	6.7%	1.8 – 22.1	3,000 – 65,000
Any STH	**2,756**	**26.2%**	**4.6 – 39.0**	**9,000 – 135,000**

LAC: Latin America and the Caribbean; DALYs: Disability-adjusted life years

In a recent review conducted by PAHO, STH prevalence data were obtained from a total of 120 studies carried out in the region (PAHO, 2011). Table 5 summarizes STH prevalence for several LAC countries. Interestingly, of 35 countries, prevalence data was available for only 18, of which eight had recently completed national level parasitological surveys. In terms of data availability, the reviewers highlighted the scarcity of information on intensity of infection in LAC for the period 2000-2010, crucial information to evaluate expected morbidity in children and to monitor effectiveness of interventions. Another important finding of this review was that one-third of the studies reflected prevalences between 20 – 50% and over one-fourth above 50%. Studies from Mexico and Venezuela were the only countries with prevalence figures < 20% (PAHO, 2011).

Table 5. Prevalence of STH infections in Latin America and the Caribbean (adapted from PAHO, 2011)

Countries with recent national surveys	**Prevalence range (%)**	**Countries without recent national surveys**	**Prevalence range (%)**
Argentina	9.0 - 38.7	Bolivia	4.5 - 65.4
Belize	43.6 - 52.2	Colombia	10.7 - 49.3
Brazil	2.0 – 36.0	Cuba	4.5 - 47.3
Haiti	15.0 – 87.0	Dominican Republic	5.3 - 55.3
Honduras	**12.2 – 97.0**	Ecuador	28.5 – 71.0
Mexico	0.01 - 16.3	Guatemala	12.7 – 68.0
Nicaragua	27.0 – 80.0	Guyana	12.3 – 38.0
Venezuela	3.0 – 19.0	Peru	1.8 - 80.4
		Saint Lucia	35.0 – 45.0
		Suriname	36.0 – 43.0

2.4b: Honduras

Honduras is among 30 LAC countries endemic for STH infections (Table 5). According to PAHO, the national average for prevalence of STH infections in Honduras is > 20% (as depicted in Figure 8) and hence these infections are a public health problem in the country. However, prevalence studies for STH infections in Honduras are scarce and fragmented. In the last 20 years only 29 studies have been published, nine of these indexed in international journals and 20 in local journals, which make them difficult to access. Unfortunately only four of these studies used Kato-Katz and thus, in some way, are comparable with the present study (Kaminsky, 1997; Kaminsky et al., 2000; Kaminsky and Retes, 2000; Smith et al., 2001). All four studies were conducted in the 90's before widespread implementation of the national deworming program and hence may not reflect the current situation.

The first paper reports a study conducted in 1993 enrolling 98 children from a suburban area in Tegucigalpa. A prevalence of 38% of *A. lumbricoides* was obtained. The study did not include diagnosis of other STH species (Kaminsky, 1997). The second paper refers to a study conducted in 1996 enrolling 94 children living in Santa Ana, a rural area of the department of Francisco Morazán. STH infections were investigated and prevalences of 22% and 19% for *Ascaris* and *Trichuris,* were obtained, respectively. No hookworm infections were detected (Kaminsky et al., 2000). The third paper reports a study conducted in 1996 enrolling 72 children living in Amapala, department of Valle. Prevalences for *Ascaris*, *Trichuris* and hookworms were 18%, 19.4% and 0%, respectively (Kaminsky and Retes, 2000). Lastly, the fourth paper reports a community-based study conducted in 1998 in four rural areas of the department of Francisco

Morazán. A total of 240 participants (children and adults) were enrolled. Prevalences for *Ascaris* and *Trichuris* were 45% and 38%, respectively. Hookworm infections were not assessed (Smith et al., 2001).

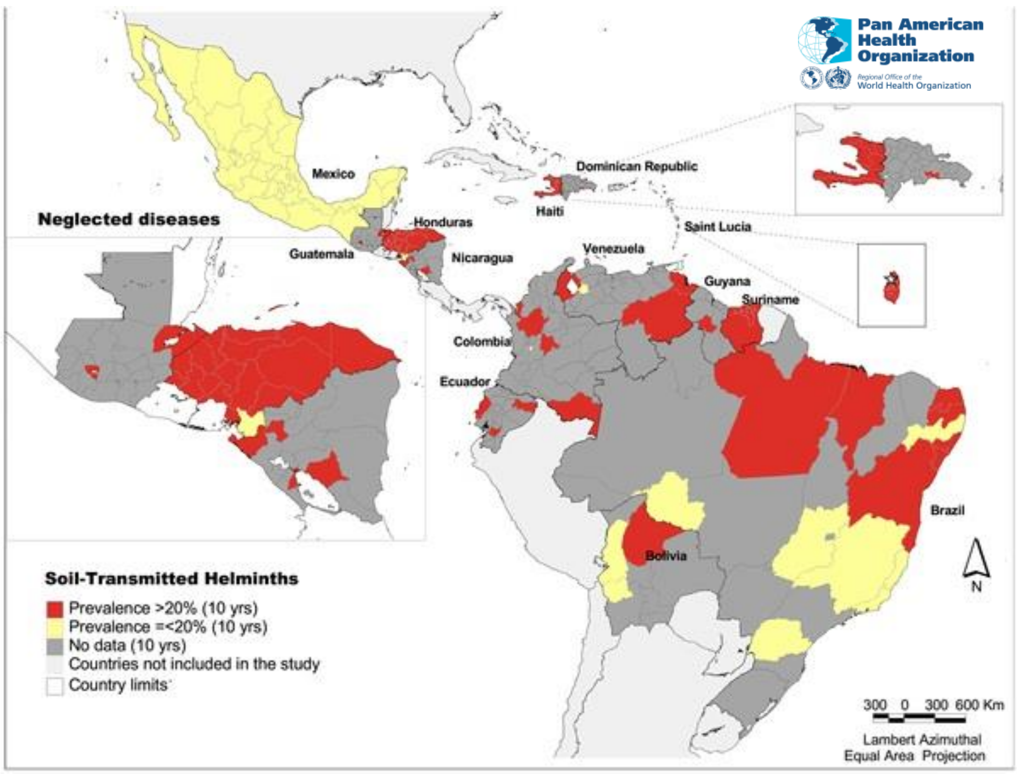


Figure 8. Prevalence of soil-transmitted helminths according to existing studies, LAC, 1998-2007. (PAHO, 2011)

Aligned with WHO/PAHO guidelines, the Honduran Ministry of Health has conducted in the last 10 years three national STH surveys using the Kato-Katz technique. The first survey was in 2001, a total of 1,197 children living in 17 communities representing eight different departments were assessed for intestinal parasites.

Prevalences of 36.5%, 51.7% and 15.7% were obtained for *Ascaris*, *Trichuris* and hookworms, respectively (Ministry of Health Honduras, 2003). The second survey conducted in 2005 estimated national prevalences of 33.7%, 42.3% and 5.3% for *Ascaris*, *Trichuris* and hookworms, respectively. This survey included 846 children from 16 rural communities. Similar to the previous survey, the sample represented eight departments (Ministry of Health Honduras, 2006). More recently, in 2011 a third national survey was conducted in 2,455 children from a total of 42 communities from all 18 departments. National prevalences of 22.9%, 35.2% and 0.9% were obtained for *Ascaris*, *Trichuris* and hookworms, respectively. Additionally, this last survey investigated co-infection of STH with malaria but no cases were found (Ministry of Health Honduras, 2011).

2.5: Health and economic burden of STH infections

According to the latest WHO estimates, more than 2 billion people worldwide are infected with soil-transmitted helminths, accounting for up to 40% of the global morbidity from infectious diseases (WHO, 2012).

In endemic populations, most infected individuals in a community will harbour light or moderate infections whereas only few will be heavily infected. The latter, however, are the major contributors to STH transmission in a community (WHO, 2002). While the most dramatic effects on health are seen in heavily infected individuals, chronic light infections and/or polyparasitism have also a detrimental effect on health and physical development, particularly in children and pregnant women (Bethony et al., 2006; Brentlinger et al., 2003; Ezeamama et al., 2005; Jardim-Botelho et al., 2008; Mupfasoni et al., 2009; Pullan and Brooker, 2008; Stephenson et al., 2000). Moreover, due their ability to cause immune-modulation in the host allowing survival of the parasite in a "modified Th2" environment, STH infections can increase the risk and severity of HIV/AIDS, malaria and tuberculosis (Behnke et al., 1992; Geiger et al., 2011; Hewitson et al., 2009; Hotez et al., 2006a; Maizels and Yazdanbakhsh, 2003). In fact, integrating STH control as a strategy for reducing the burden of malaria and HIV/AIDS has been proposed and merits deeper consideration (Hotez et al., 2006b; Molyneux, 2008; Weaver et al., 2010).

As explained previously, due to their chronic and insidious nature STH infections cause more disability than death. The overall disease burden can be assessed using the disability-adjusted life year (DALY), expressed as the number of years lost due to ill-health, disability or early death (Bethony et al., 2006; WHO, 2012). As shown earlier in Table 4, the annual global mortality caused by STH infections has been estimated at about 135,000 deaths, a seemingly low proportion from the 2 billion of persons infected with STH. Conversely, STH infections account for about 40 million DALYs globally; more than half of these caused by hookworm infections. In terms of parasitic infections, this burden is only slightly surpassed by malaria with 46.5 million DALYs. In Latin America and the Caribbean, the toll of STH infections has been estimated at 4 million DALYs (Hotez et al., 2008).

The low mortality rate of STH infections should not be erroneously interpreted as of low public health importance. As mentioned before, morbidity caused by soil-transmitted helminthiases leads to poor school performance and school absenteeism in children, as well as adverse pregnancy outcomes and reduced work productivity in adults

(Guyatt, 2000). In countries where the connection between STH infections and economic prosperity is not well understood, these infections trend to be neglected by public health programs (PAHO, 2007). The links between poverty and STH are complex and work in both directions. High prevalence of STH infections is one of the several contributing factors to poor economic growth. In turn, the poor economic growth usually entails poor level of sanitation and high prevalences of soil-transmitted helminthiases (de Silva et al., 2003). In short, these infections are consequences of poverty and at the same time are a significant cause of poverty (Schneider et al., 2011).

2.6: Prevention and control of soil-transmitted helminthiases

According to WHO, a comprehensive control program should include three major interventions: a) periodic deworming, b) health education and c) improvement of water and sanitation (Montresor et al., 2011; WHO, 2010). Periodic deworming reduces the worm burden and, therefore, the morbidity associated with STH infections producing noticeable improvements in health (Adams et al., 1994; Cooper et al., 2006; de Silva, 2003; Northrop-Clewes et al., 2001; Stephenson et al., 1989; Stoltzfus et al., 1997; Watkins and Pollitt, 1996; WHO, 2006). By reducing the worm burden, periodic deworming also reduces the number of eggs passed in the stool and, therefore, contributes to interrupt the parasites' cycle. The latter, however, cannot be achieved without an effective and efficient sanitation infrastructure along hygienic practices in the population (WHO, 2012). By increasing health education, creating awareness and addressing people's behaviours, health and hygiene promotion reduce transmission and reinfection (Aiello et al., 2008; Asaolu and Ofoezie, 2003; Ekpo et al., 2008; Fewtrell et al., 2005; WHO, 2006). Finally, by reducing contact with fecally contaminated soil or water, improvements in sanitation and access to clean water decrease transmission of STH infections (Corrales et al., 2006; Mara et al., 2010; Soares Magalhaes et al., 2011; WHO, 2006; Ziegelbauer et al., 2012). Only through the integration of these three key interventions long-term control and elimination of STH infections can be assured (Tchuem Tchuente, 2011). However, as a result of particular epidemiological situations and resources availability, the improvement in sanitary conditions, the provision of health education and access to safe drinking water are construed as more complex and long term goals. Indeed, it is argued that interventions that only take into account sanitation and changes in defecation practices may yield positive results after years or decades (Molyneux et al., 2005). Conversely, periodic deworming is conceived as a less complex, affordable, cost-effective first line of intervention (Albonico et al., 2008; Crompton et al., 2003; Molyneux et al., 2005; Montresor et al., 2011; WHO, 2012).

For these reasons, the Fifty-fourth World Health Assembly in the resolution WHA54.19 adopted in 2001, urged Member States to ensure access to anthelmintic drugs in all health services in endemic areas, to treat clinical cases and provide treatment to high-risk groups such as school-age children and pregnant women. Additionally, the resolution addressed the need for improvement in sanitary conditions, access to safe water and increased awareness through health education. This resolution recommended member states to achieve a minimum target of mass deworming of at least 75% of school-age children at risk of morbidity from STH and schistosomiasis by 2010 (WHO, 2001). Since 2006, following the strategy advocated by WHO and other partners, global control efforts rather than aiming at elimination have focused on decreasing transmission and reducing morbidity (defined as the elimination of infections of moderate and heavy intensity), especially in high-risk groups (WHO, 2012). The main basis of these efforts lay on preventive chemotherapy (PC) as explained below.

2.6a: Preventive chemotherapy (PC)

In the context of STH, preventive chemotherapy is the use of anthelminthic drugs, either alone or in combination, as a public health tool against helminth infections. As shown in Table 6, there are four drugs recommended by WHO: albendazole, mebendazole, levamisole and pyrantel pamoate. Of these, the most commonly used are albendazole and mebendazole since they are more effective and can be easily administered in single doses (400 mg or 500 mg, respectively) making them more suitable for mass deworming programs (Olliaro et al., 2011; WHO, 2006, 2012).

Table 6. Anthelminthic drugs recommended for treatment of STH infections (adapted from WHO, 2006)

STH species	Albendazole	Mebendazole	Levamisole	Pyrantel
A. lumbricoides	✓	✓	✓	✓
T. trichiura	✓	✓	✓[a]	✓[a]
Hookworms	✓	✓	✓	✓

✓ Recommended by WHO
[a] Levamisole and pyrantel have only limited effect on *T. trichiura* infections, but in combination with oxantel, pyrantel has an efficacy against trichuriasis comparable to that observed with mebendazole.

Although albendazole and mebendazole have been claimed as very efficacious against STH infections (WHO, 1999), several studies have demonstrated that, when administered in single dose, the efficacy is highly variable. It is widely recognized that this regimen is more efficacious for *Ascaris* and hookworm infections than for *Trichuris* infections. Vercruysse et al. (2011) reported the results from trials undertaken in seven countries among schoolchildren, assessing the efficacy of single-oral 400 mg dose of albendazole against STH infection. The overall cure rates (CR) obtained were 98.2% for *Ascaris*, 87.8% for hookworms and 46.6% for *Trichuris* (Vercruysse et al., 2011b). These findings are consistent with results of a meta-analyses in which overall CR for single dose of 400 mg albendazole were 93.9%, 78.4% and 43.6% for *Ascaris*, hookworms and *Trichuris*, respectively (Keiser and Utzinger, 2008). These data raise two important concerns: ineffective control of *Trichuris* infections and the possibility of resistance to anthelmintic drugs. Resistance to anthelmintics has been largely studied in veterinary nematodes (Prichard, 1990). The widespread and frequent use of anthelmintic drugs has led to development of resistance. Particularly for albendazole and other benzimidazoles, resistance has been mostly associated to a single nucleotide polymorphism (SNP) which causes an amino acid substitution from phenylalanine (Phe, TTC) to tyrosine (Tyr, TAC) in parasite β-tubulin at codon 200, reducing its affinity to benzimidazoles. (Barrere et al., 2012; Good et al., 2012; Hodgkinson et al., 2008; Prichard, 2007). However, studies of benzimidazole resistance in human helminths are scarce and their results are not conclusive (Albonico et al., 2004; Diawara et al., 2009; Geerts and Gryseels, 2001; Vercruysse et al., 2011a); therefore, its current situation has yet to be elucidated. If resistance against albendazole and mebendazole occurs, it will be a major threat to the mass deworming programs in developing countries (Diawara et al., 2009). It is quite important to continue assessing anthelmintic drugs' efficacy using standardized and validated protocols in order to make these studies comparable so proper decisions can be made (Horton, 2011).

In Honduras, two thirds of children aged 1–14 years require preventive chemotherapy (PC) for STH (WHO, 2012). Indeed, the Preventive Chemotherapy and Transmission Control (PCT) databank of the WHO estimates that 2.6 million Honduran children (769,405 pre-school and 1,832,476 school-age children) are at risk for STH transmission therefore requiring regular PC administration (WHO PCT databank, 2013). Organized STH control activities in the country (mainly based on PC) began in 1998 with the establishment of the Healthy Schools Program, a collaborative effort between the ministry of health and the ministry of education and the World Food Program (Ministry of Health Honduras, 2011). By 2001, Honduras had started subnational control activities (Ministry of Health Honduras, 2011; WHO, 2012) and these soon evolved into a national program guided by the recommendations outlined in the WHA 54.19 (WHO, 2001).

2.7: Epidemiology and risk factors

As discussed earlier, STHs have a direct life cycle without needing an intermediate host. Humans are infected by ingestion or contact with fecally contaminated soil, food or water. In general, STH's eggs are very resistant to environmental factors and their transmission and distribution rely on favorable conditions, which allow parasite species to survive, proliferate and perpetuate. However, rather than simply depending on climate and soil, these favourable conditions, as depicted in Figure 9, are in fact a dynamic and complex matrix involving biological, socio-economic and behavioural factors (Weaver et al., 2010). Each of these factors is dynamically changing and evolving over time through interactions with each other (Gazzinelli et al., 2012).

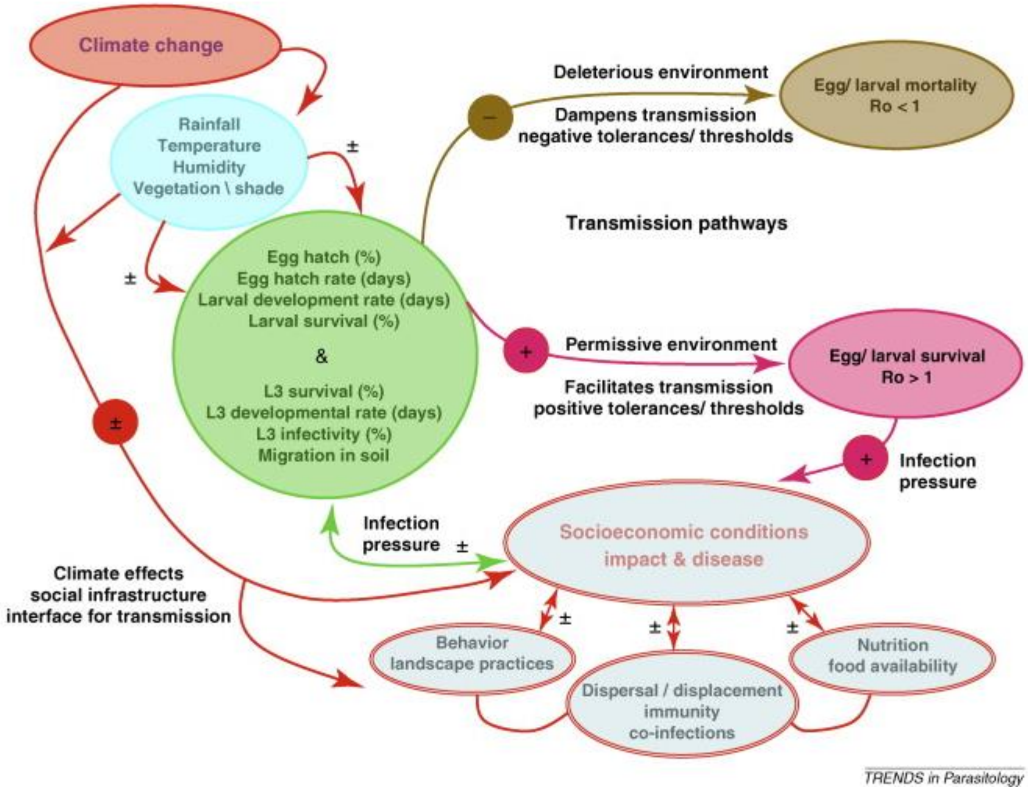


Figure 9. Biological development of STHs, showing the complexity of interactions between potential climatic and socioeconomic factors that will serve as determinants of distribution and disease. (Weaver et al., 2010)

From a simplistic perspective, it can be stated that STH infections are rooted in poverty and the corresponding lack of sanitation (Brooker et al., 2006; Hotez et al., 2008; Montresor et al., 2011; Weaver et al., 2010; WHO, 2012). However, there are significant variations both in prevalence and infection intensity within countries and within communities. It follows then, that other factors permitting or hindering STH transmission and their impact in human health might be at play. In order to achieve an effective and stable control of these infections, it is necessary to better understand the dynamics of transmission, particularly risk factors operating in endemic communities (Lustigman et al., 2012; Weaver et al., 2010).

Several studies have looked for possible associations between STH infections and putative risk factors, trying to provide better insights on this regard. Due to the focus of the present study, major emphasis on those studies conducted in schoolchildren was made. A summary of key findings in such studies is presented in Table 7. For descriptive purposes, risk factors were grouped into five categories:

a) host biology,

b) host behaviour,

c) socio-economic,

d) environmental and

e) other factors.

Table 7. Literature review of recent studies examining putative risk factors for soil-transmitted helminthiases in schoolchildren

Category	Risk factor	No association found	Association found[a]: Any STH	*Ascaris*	*Trichuris*	Hook-worms	References	
Host biology	Age	(2, 4, 7, 8, 12, 13, 14, 16)	*	*	*	(1, 9, 11, 15)[b]	1	Alemayehu, 2008
	Sex	(2, 3, 10, 12, 13, 16)	(4:M, 7:F)	*	*	(1, 6, 8, 9, 15: M)	2	Alemu et al., 2011
Host behaviour	Lack or low frequency of handwashing	*	(1, 2)	*	*	*	3	Ambumani & Mallika, 2011
	Not wearing shoes	(6)	*	*	(8)	(2)	4	Ekpo et al., 2008
	Open defecation	(1, 2)	*	*	*	*	5	Fouamno Kamga et al., 2011
Socio-economic	Low mother's education	(2, 7)	*	(6, 13)	(6, 9)	(6, 9, 15)	6	Gunawardena et al., 2011
	Lack of health education	*	(5)	*	*	*	7	Hesham Al-Mekhlafi et al., 2008
	Low socio-economic status	(7)	*	*	*	*	8	Lello et al., 2013
	Low family income	(10)	*	*	*	*	9	Mofid et al., 2011
Environ-mental	Cluster effect	*	*	(8, 14)	(8, 14)	(15)	10	Naish et al., 2004
	Lack of access to safe water	(7)	(1, 2, 12)	*	*	(15)	11	Odiere et al., 2011
	Lack of toilet/latrine in house	(16)	(7, 12)	*	*	(15)	12	Scolari et al., 2000
	Owning domestic animals	(7)	*	*	*	(15: pigs)	13	Sorensen et al., 2011
Others	STH Co-infection	*	*	(8: *Trichuris*)	(8: *Ascaris*)	(8: *Trichuris*)	14	Standley et al., 2009
	Low frequency of deworming	*	*	(6)[c]	*	*	15	Steenhard et al., 2009
							16	Yami et al., 2011

STH: soil-transmitted helminth; F: Female; M: Male

[a] Statistical association with $p < 0.05$ calculated by either univariate or multivariable analyses

[b] Risk of hookworm infections increased with age

[c] Risk of Ascaris infections decreased when frequency of deworming increased

[*] Not reported

2.7a Host biology

Age. Although not entirely understood, it is well known that there is an age-dependant pattern in STH prevalence and intensity of infection occurring in endemic communities. This is particularly true for *Ascaris* and *Trichuris* infections. This pattern exhibits a rise with age in childhood, typically peaking within 6 and 10-years of life. In adults, in contrast, even if prevalence remains high in adulthood, intensity typically declines, as shown in Figure 10a (Anderson et al., 1992; Bundy and Cooper, 1989; Dold and Holland, 2011; Galvani, 2005; Holland, 2009; Hotez et al., 2006a; Scott, 2008). This has been explained as resulting from a reduced exposure to eggs, an accumulated immunological resistance to incoming infection, or a combination of both (Bogitsh et al., 2012; Faulkner et al., 2002; Galvani, 2005; Hotez, 2008a). For hookworms however, maximum prevalence is usually reached in adolescence and early adulthood and is generally associated with certain occupations with increased exposure to contaminated soils (Nelson and Masters, 2007). Age-intensity profile for hookworms shows large variation. As illustrated in Figure 10b, the most commonly recognized pattern is a steady rise in the intensity of infection during childhood, with either a peak or a plateau in adulthood (Brooker et al., 2004; Hotez et al., 2004).

However, elucidating age-related differences in prevalence of STH infections in school children has proved more difficult, maybe due to the narrow age range of the research participants (Alemu et al., 2011; Ekpo et al., 2008; Hesham Al-Mekhlafi et al., 2008; Lello et al., 2013; Scolari et al., 2000; Sorensen et al., 2011; Standley et al., 2009; Yami et al., 2011). In 1998 in India, Naish and co-workers found that intensity of ascariasis was significantly lower in children ≥ 9 years old compared to younger children

(Naish et al., 2004). Additionally, a few authors have reported that the risk of hookworm infections significantly increased with the age of the studied population (Alemayehu, 2008; Mofid et al., 2011; Odiere et al., 2011; Steenhard et al., 2009).

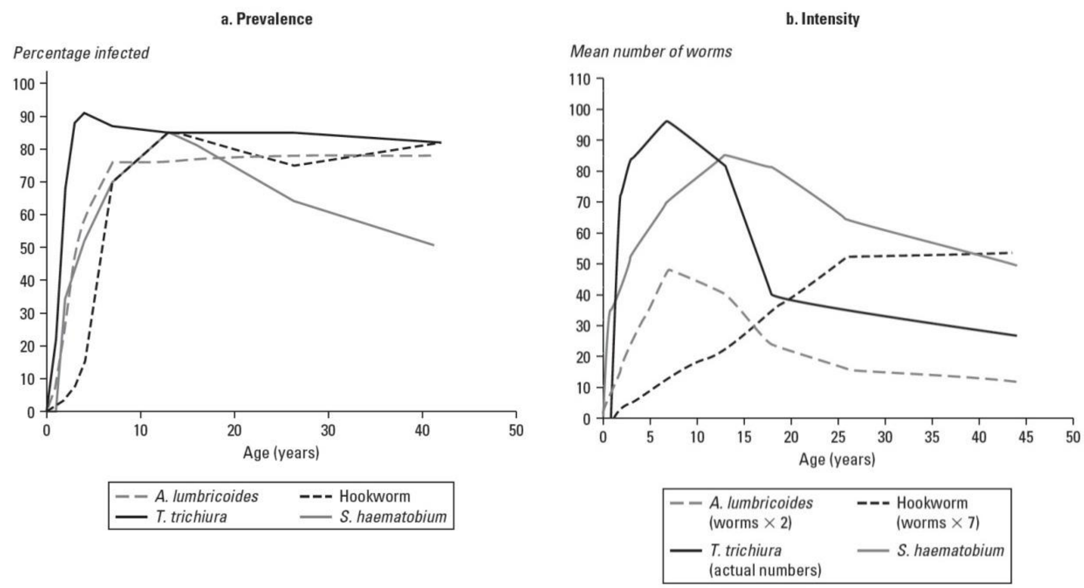


Figure 10. Age-associated prevalence and intensity profiles of STH and *Schistosoma* infections: typical age profiles of prevalence and intensity of STH infections and Schistosomiasis. (Hotez et al., 2006)

Sex. While *Ascaris* and *Trichuris* infections have not been associated with sex, prevalence and intensity of hookworm infections are typically higher in men than in women (Brooker et al., 2004; Bundy, 1988; Hotez et al., 2004). Researchers have suggested that this phenomenon may be caused by differential exposure (males and females perform different activities), physiologic differences between males and females (steroid hormones as testosterone and progesterone can modulate the immune response), or a combination of both (Moore and Wilson, 2002; Nava-Castro et al., 2012; Poulin, 1996; Zuk and McKean, 1996). Noteworthy, few studies in Asia have reported higher intensity of hookworm infections in women than in men (Bethony et al., 2002; Gandhi et al., 2001; Needham et al., 1998).

Several studies conducted in school children have found that boys were more likely to harbour hookworm infections than girls of the same age (Alemayehu, 2008; Gunawardena et al., 2011; Lello et al., 2013; Mofid et al., 2011; Steenhard et al., 2009), while in others, no significant differences could be found (Alemu et al., 2011; Anbumani and Mallika, 2011; Naish et al., 2004; Scolari et al., 2000; Sorensen et al., 2011; Yami et al., 2011).

2.7b Host behaviour

Lack or low frequency of handwashing. Since fecal contamination is the most important form of transmission of STH, a good hygiene and periodic handwashing using soap is recommended as a preventive measure (Montresor et al., 2011). However, as described in the systematic review performed by Fung and Cairncross (2009), the empirical evidence supporting this association is both scarce and weak. This review identified one intervention study, conducted by Chen and Xu, in a cohort of 336 Chinese primary school students in 2005. All children were positive for *Ascaris*, then treated with albendazole and confirmed negative using Kato-Katz. Re-infection with *Ascaris* was studied. Children in control group had no intervention. Children in intervened group were asked to wash their hands with soap before meals, after defecation or when their hands get dirty, and to keep a record of their handwashing. After one-year follow-up, the intervened group had only 44% probability of harbouring *Ascaris* infections compared to the control group (Fung and Cairncross, 2009).

Additionally, a couple of cross-sectional studies conducted with Ethiopian schoolchildren were able to identify handwashing as a protective factor for STH infections (Alemayehu, 2008; Alemu et al., 2011). The study conducted by Alemayehu used multivariable analysis controlling for possible confounding and their findings are therefore more robust. Alemayehu's findings are more comparable with the present study.

Not wearing shoes. By preventing skin (bare foot) to be in contact with infective hookworm larvae, wearing shoes has been suggested since the early 20th century as an effective way to reduce hookworm infections (Bleakley, 2007; Hotez, 2008c; Smillie, 1924). However, scientific evidence remains inconclusive. Alemu and co-workers (2011) reported that wearing shoes was a protective factor against hookworm infections in Ethiopian schoolchildren. Lello and colleagues (2013) reported a similar result against trichuriasis in a study conducted in Zanzibar. Conversely, a study carried out by Gunawardena and collaborators in Sri Lanka could not demonstrate statistical association between wearing shoes and hookworm infections (Alemu et al., 2011; Gunawardena et al., 2011; Lello et al., 2013).

Open defecation. Considering the lifecycle of STH, it is evident that open defecation (OD) plays a major role in the transmission of these parasites, as it leads to soil contamination and widespread exposure to infective eggs. Interestingly, OD practice *per se* has not been significantly associated with increased likelihood of infection in the same individual (Alemayehu, 2008; Alemu et al., 2011). Whether the level of endemicity or methodological issues can explain this lack of association remains to be elucidated.

2.7c Socio-economic

Low mother's education. Several studies have assessed the impact of maternal education in children's STH infection. Higher level of maternal education usually lead to higher awareness and better hygiene practices which would prevent these parasitic infections from occurring, therefore lower level of maternal education is considered a risk factor for children's STH infections (Brocklehurst and Bartram, 2010). Lower maternal education of school children has been indicated as a significant predictor for ascariasis (Gunawardena et al., 2011; Sorensen et al., 2011), trichuriasis (Gunawardena et al., 2011; Mofid et al., 2011) and hookworm infections (Gunawardena et al., 2011; Mofid et al., 2011; Steenhard et al., 2009). However, this association has not been always demonstrated (Alemu et al., 2011; Hesham Al-Mekhlafi et al., 2008).

Inadequate health education. Community-based health education has been highly recommended by WHO as one of the three major components of comprehensive STH control programs (Montresor et al., 2011; WHO, 2010). Encouraging evidence comes from an intervention study conducted in 370 schoolchildren (208 in intervention group and 162 in control group) from the South-West Region of Cameroon. Health education was given to the intervention group, promoting and reinforcing health behaviour; encouraging particularly those aspects of personal hygiene pertinent to the control of fecal-orally transmitted parasitic infections. Six month after the intervention, both groups were assessed for STH and other intestinal parasites. When comparing STH prevalences before and after health education intervention, no significant difference was observed in the group control (51.5% vs. 57.5%), whereas a significant decrease in STH prevalence (50.9% vs. 26.9%) was noticed in the intervention group (Fouamno Kamga et al., 2011).

<u>Low socio-economic status</u>. In Latin America and the Caribbean, poverty is a major determinant for STH infections (Hotez et al., 2008). Therefore, individual's socio-economic status (SES) and the level of community development are important drivers for STH transmission and reinfection. However, due to its dimensionality, measuring poverty is very complex (Deininger and Squire, 1996). Several methods are used to assess poverty but no consensus exists as to which one better captures the level of poverty in individuals, regions or nations (Boltvinik, 1998). In general, three methods are frequently used: a) asset-based, b) income-based and c) unsatisfied basic needs (UBN). The first two measure the economic dimension of wellbeing, whereas the latter also ascertains physiological and socioeconomic resources (Hammill, 2009). In Honduras UBN is the common method utilized to assess poverty (Macias Ruano et al., 2009) but for the present study the asset-based method will be used. The reason for this predilection is that this approach has proved very useful in STH studies worldwide.

Basically, an asset-based approach utilizes proxy variables which, after further analyses, are converted into factors representing the SES of the study population (McKenzie, 2005; Montgomery et al., 2000; Raso et al., 2005). The amount and nature of the proxy variables will depend on the societal context of the study. For example, some studies may use owning a car, a motorcycle or a telephone while others may use owning a bicycle or having access to drinking water or owning domestic animals. This broad approach to assessing assets demonstrates that poverty can be a relative concept.

Some studies conducted in different populations (adult and children) have reported a significant association between SES and prevalence of STH infections. Higher prevalences of any STH (Balen et al., 2011), ascariasis (Conlan et al., 2012; Hohmann et al., 2001; Kounnavong et al., 2011; Sayasone et al., 2011), trichuriasis (Conlan et al., 2012; Halpenny et al., 2013) and hookworm infections (Halpenny et al., 2013) were observed in individuals with lower SES. Conversely, this association could not be identified in a community-based study conducted in El Salvador (Corrales et al., 2006) nor in a longitudinal study conducted in Malaysia with 120 school children (Hesham Al-Mekhlafi et al., 2008).

2.7d Environmental

Cluster effect. Communities, even when physically close to each other, can differ in development level, environmental conditions and cultural practices. As mentioned, all these factors can affect STH transmission in a particular community. Due to a similar mechanism of transmission, *Ascaris* and *Trichuris* infections have been more associated with a cluster effect (Conlan et al., 2012; Gamboa, 2011; Lello et al., 2013; Standley et al., 2009) than hookworm infections (Steenhard et al., 2009). In 2002, Nishiura and co-workers reported that the intensity of *Ascaris* infections in schoolchildren varied significantly across villages in spite of villages' similarities in economic development, environmental conditions and inhabitants' level of education. The authors speculate that such differences might have been caused by particular health interventions that took place prior their study (Nishiura et al., 2002).

Lack of access to safe water and sanitation. Hygiene, sanitation and safe water have been described as "forgotten foundations of health" (Bartram & Cairncross, 2010) and, according to some reviews and meta-analyses, inadequate water, sanitation and hygiene are independent risk factors for major diarrhea-causing infections and parasitoses such as intestinal helminthiases (Clasen et al., 2007; Fewtrell et al., 2005; Mara and Feachem, 1999; Ziegelbauer et al., 2012). Some studies have associated good quality water with low STH prevalences in school children (Alemayehu, 2008; Alemu et al., 2011; Scolari et al., 2000; Steenhard et al., 2009), although this association has not been always demonstrated (Hesham Al-Mekhlafi et al., 2008).

Similar conflicting results have been obtained when assessing the association between lack of sanitary facilities and STH infections. Some studies have reported lower STH prevalences in children who have sanitary facilities (*i.e.*, latrine or toilet) available at home (Hesham Al-Mekhlafi et al., 2008; Scolari et al., 2000) while in others, this association could not be demonstrated (Yami et al., 2011). Additionally, Ekpo and co-workers (2008) reported that school children attending public schools in Nigeria had higher STH prevalence than those attending private schools with much better facilities and hygienic conditions (Ekpo et al., 2008).

Owning domestic animals. The idea of zoonotic transmission of STH is surrounded by controversy but human infections with porcine and canine species of STH have been reported.

Ascaris suum, the swine roundworm, is globally distributed and can be found in most porcine production systems. There is a potential risk of cross-infections when living in close proximity with pigs or when using pig manure as fertilizer on vegetables for human consumption (Nejsum et al., 2012). In developed countries where *Ascaris lumbricoides* is unusual, *Ascaris suum* has been implicated in human ascariasis, for example in Denmark (Nejsum et al., 2005; Roepstorff et al., 2011) and the United Kingdom (Bendall et al., 2011).

Several cases of human infections with the canine species, *Trichuris vulpis,* have been reported. However, doubts remain since diagnosis has been done on the basis of egg morphology (*T. vulpis* are about twice the size of *T. trichiura* eggs) and no one has conducted molecular analysis (Traversa, 2011). Interestingly, even when more zoonotic potential lies with the porcine species, *Trichuris suis*, no cases have been reported so far (Nejsum et al., 2012). However, in the middle 1970's Beer experimentally demonstrated for the first time that cross-infection in both ways, *T. suis* in man and *T. trichiura* in pigs, was possible (Beer, 1976). In El Salvador, Corrales and co-workers (2006) found a significant association between owning pigs and prevalence of *Ascaris* and hookworm infections (Corrales et al., 2006). Additionally, Steenhard and colleagues (2009) reported a similar association between hookworm infections and pigs in schoolchildren in Guinea-Bissau (Steenhard et al., 2009)

One canine hookworm species, *Ancylostoma ceylanicum*, is able to complete its cycle in humans but it is not considered an important pathogen (Hotez et al., 2004).

Recent studies in Southeast Asia suggest, however, that this problem may have been overlooked (Conlan et al., 2012; Mahdy et al., 2012; Ngui et al., 2012).

In Malaysia, Ngui and colleagues (2012) investigated hookworm prevalence in 634 human and 105 domestic dogs and cats. Species were identified using molecular analysis. In humans, they found that most of the infections were caused by *Necator americanus*. However, *A. ceylanicum* accounted for 12.8% of single infections and 10.6% mixed infections. On the other hand, *A. ceylanicum* accounted for 52% of dogs' and cats' infections. Additionally, hookworm infections were significantly associated with close contact with pets (Ngui et al., 2012). In another Malaysian investigation with 221 dogs, *A. ceylanicum* accounted for 52.4% of the infections and prevalences were significantly higher in stray dogs. Perhaps the more interesting finding of this study is that, after phylogenetic analyses, *A. ceylanicum* sequences isolated from dogs and humans (Ngui et al., 2012) were strongly grouped together (Mahdy et al., 2012). In 2009, Conlan and colleagues conducted a community cross-sectional survey of STH in human and dogs in four provinces in Laos exposed to mass deworming. In this study, *A. ceylanicum* constituted 17.8% of the hookworm infections in humans. More interestingly, 22.3% of the dogs were infected with *Necator americanus*, the human hookworm (Conlan et al., 2012).

2.7e Other factors

<u>STH co-infection</u>. In developing countries, polyparasitism is the rule rather than exception (Raso et al., 2004; Steinmann et al., 2010) but very few studies have focused on possible associations among STH species. Using multivariable analyses, Lello and colleagues (2013) demonstrated that significant increase in prevalence for all three STH was associated with co-infection state. Infection with *T. trichiura* was associated with higher prevalence of *A. lumbricoides* and hookworm infections, whereas co-infection with *A. lumbricoides* was associated with higher prevalence of *T. trichiura* infection (Lello et al., 2013). Conversely, no significant association was found in a community-based study conducted in Colombia (Agudelo-Lopez et al., 2008)

Frequency of deworming. Preventive chemotherapy (PC) is a cost-effective and easy-to-implement strategy for eliminating morbidity associated with STH (Albonico et al., 2008; Molyneux et al., 2005; Montresor et al., 2011; WHO, 2012). Frequency of PC is determined by the overall STH prevalence. Regions with prevalence ≥ 50% required deworming 2 - 3 times a year (WHO, 2012). Schoolchildren are one of the main target groups for this type of interventions. However, few research studies have been done to determine the reduction on STH prevalence after PC activities. In a study conducted in 4th grade children in Sri Lanka, the frequency of PC was significantly associated with a reduction in *Ascaris* infections (Gunawardena et al., 2011). The health benefit of deworming has received attention but the evidence has not been consistent. A recent longitudinal study from India showed that regular deworming has little effect in childhood mortality (Awasthi et al., 2013). A meta-analysis conducted by Taylor-Robinson and co-workers (2007) evaluating the effect of deworming on growth and school performance in children, concluded that research evidence is not sufficient to recommend deworming drugs in targeted communities (Taylor-Robinson et al., 2007). This conclusion has been met with criticism by PC proponents. They consider that the observed inconsistency only reflects methodological issues in the reviewed studies. They argue that these studies were looking only for biomedical outcomes, and that other benefits of PC in children such as the effect of deworming increasing primary school participation, and the respective implication for the future development of the individual and society, should be also considered (Bleakley, 2007; Bundy et al., 2009).

2.7f Risk factor studies conducted in Honduras

No peer-reviewed publications assessing risk factors for STH infections in Honduran schoolchildren could be found upon literature search and therefore, no point of reference exists for the present study. In 1998, a community-based study conducted by Smith and co-workers (2001) assessed the prevalence of *Ascaris* and *Trichuris* infections in four rural Honduran communities. The study also evaluated some risk factors using univariate and multivariable analyses. Parents' education, lack of latrine, open defecation, recent history of diarrhea, community and households members infected with *Trichuris* were all significant predictors for *Ascaris* infections in the univariate analysis. However, in the multivariable model only recent history of diarrhea and household members with trichuriasis remained significant. For *Trichuris* infections, lack of latrine, open defecation and household members with ascariasis were significant predictors in the univariate analysis. However, no predictor remained significant in the multivariable models. Gender was not significantly associated to STH infections (Smith et al., 2001).

Upon examining the Honduran gray literature on STH, one PhD and eight MD theses were found. All MD theses assessed STH prevalence and its association with at least one risk factor. However, due to insufficient methodological details on how these risk factors were assessed, the quality of their findings remained uncertain. Moreover, none of these studies were conducted in schoolchildren and therefore not useful for the present thesis.

In regards to the PhD thesis, authored by Ciliezar (2003), results of a nationwide survey on STH conducted in 1998 - 1999 were analyzed and the association of STH prevalence with human development was explored. Using the Human Development Index (HDI) as indicator, Ciliezar found that higher STH prevalences were associated with lower HDI values (Ciliezar, 2003).

In light of all the findings described throughout this literature review, it can be concluded that STH infections have an important detrimental effect in children's health and deserve to be investigated. Further, it was established that Honduras is lacking scientific evidence on the risk factors associated with STH infections, particularly in children. Therefore, the aim of the present study is to contribute with updated data of STH prevalence and infection intensity in a subpopulation of rural Honduran schoolchildren. More importantly, this thesis intends to provide insights into STH transmission in Honduras by conducting a risk factor analysis for these important parasitoses.

CHAPTER 3: METHODOLOGY

The present study was part of a parent study titled ‘Gender and parasitic diseases: Integrating gender analysis in epidemiological research on parasitic diseases to optimize the impact of prevention and control measures’, a collaborative effort with McGill University and local researchers in Honduras, Senegal and Ethiopia.

3.1: Study design

Both the gender and present studies were school-based, cross-sectional studies, designed as explorative and hypothesis generating studies.

3.1a: Study area and communities

This study was conducted in selected rural communities of the municipality of Catacamas in the Department of Olancho, Honduras. In terms of area, Catacamas is the largest municipality in Central America (7,173.89 km^2) and it is located in the largest department of Honduras, Olancho (24,351 km^2). In 2010, temperature in the zone ranged from 19.9ºC to 30.3ºC with an annual precipitation of 1409 mm. Its only urban setting, Catacamas city, is located in a valley at 450 meters above sea level, between 14° 51' 0'' North latitude and 85° 50' 0'' West (Meridian Greenwich). It is around 210 km north-east from Tegucigalpa, the Capital city of Honduras (Wikipedia, 2013). Due its extension and climate, the municipality of Catacamas displays an interesting mosaic landscapes and types of communities. In general, Catacamas can be recognized mainly as a forest area though having important agricultural activities framed in its tropical semi-humid climate. Livestock production represents 42% of the gross agricultural production, while corn, beans and coffee crops constitute about 80% of the gross value of crop production.

(Henry, 2009). According to the Honduran National Institute of Statistics, the estimated population of the municipality of Catacamas for 2011 was 112,909 inhabitants, 58.4% of them living in rural communities. Forty nine percent of the population of Catacamas is aged ≤ 17 years (http://www.ine.gob.hn/drupal/, accessed July 23th, 2013).

Nine rural communities were selected for this study: Colonia de Poncaya; Las Lomas de Poncaya; Las Parcelas; Corosito de Poncaya; Los Lirios; El Cerro del Vigía, El Hormiguero; Santa Clara and Campamento Viejo. Reasons for selecting these communities are as follows. Firstly, all the communities were representatives of rural Honduras; secondly, the schools in these communities were officially enrolled in the National Deworming Program; finally, all these communities were collaborating with one of our local partners, the National University of Agriculture (UNA), thus facilitating all the work and coordination with the communities.

The study communities were located about 2 hours drive from Catacamas city and accessed only by dirt roads. Due to the logistics required, team's headquarter and laboratory were established at UNA in Catacamas and hence, the research team travelled daily to the communities. Lab analyses were done at UNA. Figure 11 shows the study area and communities.

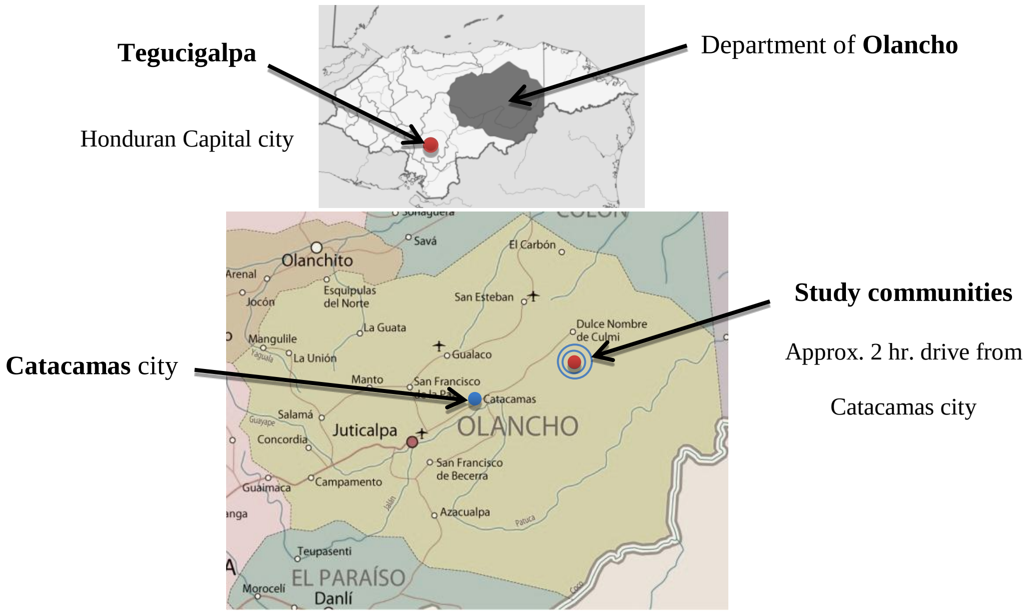


Figure 11. Study area and communities located in the Department of Olancho, Honduras.

3.1b: Study population

Both the gender and present studies targeted school-age children enrolled in grades 3rd to 5th in rural schools of the area for several reasons: a) STH infections are more frequent in children than adults (Hotez, 2008b); b) heavy infections with *Ascaris lumbricoides* and *Trichuris trichiura* are usually observed in school-age children (WHO, 2002); c) school-age children are considered at high-risk of STH infections and major targets for systematic regular treatment (WHO, 2002); and d) at this age, children are old enough to provide reliable information when interviewed for collecting basic demographical and epidemiological data.

3.1c: Sample size estimation

For the parent study, power and sample size determination were performed by Dr. Theresa Gyorkos of McGill University, QC, utilizing the PS software (version 3.0, January 2009, by William D. DuPont and Walton D. Plummer Jr.). This was based on a two-sided chi-square test. Using previous studies in Peru as a reference (Casapia et al., 2006) it was assumed that half of the children in this school-age group will be male and that the prevalence of any STH would be 50% in males. A total of 314 participants were therefore needed to detect a minimum risk ratio of 1.5 with 80% power. A response rate of at least 80% was established and, consequently, a target sample of 377 children (314+63) was targeted for enrollment. The present study was bound by this sample size determination.

3.2: Ethical approvals

The studies received ethical approval from all participating institutions, both in Canada and Honduras:

1) Brock University, St. Catharines, ON. File number - BU 10-161– Sanchez/Gyorkos Jan 13th 2011 (Appendix A).
2) McGill University Health Centre, Montreal, QC. File number - MUHC 10-175-PED Nov. 23rd 2010 (Appendix B)
3) Ethics officer, Master's degree program in Zoonotic and Infectious Diseases of the School of Microbiology, National Autonomous University of Honduras (MEIZ-UNAH), Dr. Vilma Espinoza. File number OF-MEIZ-Dictamen-001-2011 (Appendix C).

In addition to those ethical clearances, approval processes were also implemented in consultation with community leaders, schools principals and grades teachers. Moreover, since the study involved children, both parental consent and children's assent were required prior to enrollment, as described below in section 3.3.

3.3: Recruitment of research participants

3.3a: Schools' selection

A list with eligible schools, location, children enrollment, deworming program status and principal's expression of interest in participating was provided by our partner in Catacamas, UNA. Subsequently, the research team selected the schools to be approached. Basically, a primary selection was made considering: a) size of schools (priority was set on larger schools); b) location (considering travel time and accessibility) and; c) deworming status (only those schools which hadn't dewormed in the last three months were considered eligible).

3.3b: Schools' enrollment

Preliminary visits to the schools and meetings with schools' principals were held. Detailed explanations and print material about the study were provided to every school principal. Also, invitation letters to participate in the study were handed out (Appendix D). Written authorizations were obtained from school principals who accepted enrolling their schools in the study (Appendix E). Only Schools with principal's authorizations were selected for the study.

3.3c: Children's enrollment

***<u>Parental Consent</u>*.** Parents and guardians of children in grades 3-5 were invited to an informational session where the objectives of the study were fully explained, making clear the risks and benefits of participating in the study. In order to a better understand from parents and guardians, in every session, explanations were provided regarding intestinal parasites and their importance as a threat to children's health; helminths' mode of transmission, control and preventive measures; environmental issues and importance of sanitary practices. At this stage, researchers clearly stated that participation of their children in this study was absolutely voluntary and they may refuse their children's participation or, may discontinue their participation at any time without explanation, and without penalty or loss of benefits to which they were otherwise entitled. Parents / guardians were encouraged to ask questions and expose any concern they could have. The research team made sure all questions or concerns were clarified before asking parents / guardians if they consented. Parents / guardians who gave oral consent for their children to enroll in the study were provided with a 'consent package' (Appendix F). This package contained a printed issue in Spanish with: a) the same information that was explained verbally (study objectives, risks and benefits, ethical issues, etc.); b) an invitation to participate and c) a consent form for their signature.

***<u>Children's Assent</u>*.** Children, whose parents consented, were invited to participate in the study. With the collaboration of grade teachers and school principals, who served as witnesses at this stage, every child was individually approached and all details of the study were properly explained, including their voluntary participation and right to withdraw without penalty or loss of benefits to which they were otherwise entitled. Only those children who expressed assent in responding to a questionnaire and providing a stool sample were then enrolled in the study. Children assents were obtained verbally and documented through a child assent form (Appendix G). Assenting children were provided with a kit for stool sample collection.

3.4: Data collection

Demographic and epidemiological data were collected using individual interviews with the use of a pre-tested standardized questionnaire (Appendix H). One stool sample was required to determine presence and intensity of infection using the Kato-Katz method (WHO, 1991).

3.4a: Individual interviews and standardized questionnaire

Only children providing a stool sample were interviewed. In order to anonymize children's data, a unique identifier (numerical code) was assigned to every child for identifying his/her stool sample and questionnaire. Interviews were conducted individually, in private and in such a way as not to interfere with school activities. The interview time ranged from 25 - 30 minutes depending on children's ability to understand and answer the questions asked. Whether children were wearing shoes or not at the interview time was also assessed and recorded. An interview guideline (Appendix I) was provided to every interviewer for criteria harmonization.

The questionnaire was structured into different categories to facilitate its administration. Basically, questions were grouped into 9 categories as follows:

1. **Basic information.** Basic demographics such as child's name; date of birth; age and gender (questions 1 - 7 on the questionnaire).

2. **Perception and knowledge about intestinal helminths.** Related to parasites' mode of transmission and health's impact (questions 8 - 13 on the questionnaire).
3. **Community and household characteristics.** Exploring sanitary conditions and facilities; access to drinking water; level of income (indirectly evaluated through house condition and appliances); overcrowding and interaction with animals (questions 14 - 23 on the questionnaire).
4. **Hygiene practices.** Assessing type and frequency of hygiene practices such as open defecation, hand washing and others (questions 24 - 31 on the questionnaire).
5. **Children's activities.** Exploring potential exposure to STH (questions 32 - 33 on the questionnaire).
6. **Use of shoes or sandals.** Exploring increased risk for acquiring hookworm infections (questions 34 - 36 on the questionnaire).
7. **Use of health services.** Assessing health care access and health status (questions 37 - 41 on the questionnaire).
8. **History of deworming.** Assessing type and frequency of treatment received (question 42 on the questionnaire).
9. **Additional questions.** This specific set of questions was added to obtain a deeper assessment of previous infection, interaction with pigs (potential zoonotic ascariasis) and health status perception (questions 43 - 46 on the questionnaire).

3.4b: School questionnaire

A standardized school questionnaire (Appendix J) was used to assess schools' demographics, sanitary condition and deworming program's status. The assessment was done through both an interview with the school principal and by direct observation by a researcher. An interview guideline (Appendix K) was provided to every interviewer for criteria harmonization.

3.4c: Questionnaires' quality control

Questionnaires were checked for completeness and consistency by a member of the research team other than the interviewer. If missing information was detected, the interviewer was warned and asked to retrieve it. Most of the time this was possible since the children were still in the school after the interview.

3.4d: Stool collection and parasite determination

A 'stool collection kit' and instructions for collecting stool sample were provided to those children enrolled in the study. Each kit contained:

- One disposable 'chamber pot' for defecation
- One non-sterile wide-mouthed leak-proof container with cover in a plastic bag – for bringing fecal specimen to the researchers
- One wooden spatula for scooping fecal specimen into the container.

A single stool sample was collected from each child, after which they were taken to the UNA laboratory for analysis using the Kato-Katz technique (Kato and Miura, 1954; Katz et al., 1972) as recommended by the WHO (WHO, 1991). In order to facilitate the specimen preparation two basics modification were introduced: a) 3" x 2" microscope slides were used instead the traditional 3” x 1” slides and, b) larger cellophane strips were used to obtain a larger area for microscopy.

Stool samples were collected in the morning by participants and delivered to researchers at interview. Samples were kept refrigerated using a portable cooler and transported in the afternoon to the laboratory at UNA for analysis the same day. Stool samples were analyzed by trained microbiologists under the supervision of a faculty member of the parasitology section, School of Microbiology (UNAH). The time from collection to Kato-Katz smear preparations was approximately six hours. Smear preparations were examined under the microscope after the clarifying time (between 30 to 60 minutes) by members of the research team. STH eggs were identified by their characteristic features and systematically counted. Fecal egg counts (FEC) were calculated for each species of STH by multiplying the numbers of eggs by a factor of 24 in order to get the number of eggs per gram of stool (epg). Based on the epg obtained, infection intensities were classified as light, moderate, or heavy according to the WHO criteria previously shown in Table 2.

3.4e: Kato-Katz quality control

For quality control purpose, 10% of the examined smears were re-examined by the research team member with greatest experience in parasitology.

3.5: Data analysis

3.5a: Data entry

All data from both children and schools questionnaires, as well as Kato-Katz results were entered by a researcher into Microsoft Office Excel spreadsheet 2007 (Microsoft) following their respective codebooks and verified for accuracy (compared with data in questionnaires) by a different researcher. Data were cleaned by checking for errors, missing values and extreme values or outliers. The methodological approach for dealing with missing values is described below. Statistical analyses were carried out using IBM SPSS Statistics for Windows version 20.0 (Armonk, NY: IBM Corp.) and Stata 12 (College Station, TX: StataCorp LP).

3.5b: Statistical analyses

Basic descriptive statistics were performed for both categorical and continuous variables for characterizing the study population.

***Objective 1 (STH prevalence)*.** Point prevalences with 95% confidence intervals (95% CI) were calculated for overall STH infections and for each STH species. Similarly, point prevalences with 95% CI were calculated for monoparasitism and polyparasitism (multiple STH infections).

Objective 2 (Intensity of STH infections). Based on the number of eggs per gram (epg) of stool and using the thresholds established by WHO (WHO, 2002), infections for each STH species were categorized as light, moderate or heavy and point prevalences with 95% CI were calculated. To construct logistic regression models, intensity of infection was reduced to two categories, moderate-to-heavy and negative-to-light.

Reasons for this categorization are as follows. Firstly, daily variability of egg output (Walker et al., 2009) or the probability of infections in the pre-patent period (Bogitsh et al., 2012). Secondly, potential loss of diagnostic sensitivity of Kato-Katz methods using a single stool sample (Knopp et al., 2011a). Finally, the public-health importance of these infections is generally associated with higher worm burdens (WHO, 2012).

Objective 3 (Risk factors). Binary logistic generalized estimating equations (GEE) models, with unstructured correlation matrix, were used to estimate the association between odds of the outcomes and selected putative risk factors. This approach accounts for the correlation among study participants attending the same school (school clustering effect). Based on the reviewed literature, 15 variables (putative risk factors) were selected for testing their association with STH (Table 8). Based on likelihood-ratio (LR), a stepwise backward elimination of non-significant variables was used to find parsimonious models best predicting the outcomes, retaining those variables with p-values < 0.2.

The defined outcomes of interest were: a) *Ascaris* infection, b) *Trichuris* infection, c) hookworm infection, d) polyparasitism, e) moderate-to-heavy ascariasis and f) moderate-to-heavy trichuriasis. Complete data for the final models were available for 96 to 100% of the participants. Because of relatively high data completion, missing data values were not imputed.

Table 8. Putative risk factors selected for testing their association with STH

Risk factor	**Type of variable**	**Category**
Age	Continuous	Not applicable
Sex	Dichotomous	Boy / girl
SES score [a]	Compound / continuous	Not applicable
STH awareness [b]	Compound / continuous	Not applicable
Earthen floor at home	Dichotomous	Yes / no
Having sanitary facility	Dichotomous	Yes / no
Piped water at home	Dichotomous	Yes / no
Handwashing	Dichotomous	Occasionally / regularly
Open defecation	Dichotomous	Yes / no
Wearing shoes outdoors	Dichotomous	Yes / no
Domestic chores	Dichotomous	Indoor / outdoor
Recalled expelling worms	Dichotomous	Yes / no
Recalled previous deworming	Dichotomous	Yes / no
School hygienic level [c]	Compound / dichotomous	Higher / lower
School deworming regimen	Dichotomous	None-once / twice a year

SES: socio-economic status; STH: soil-transmitted helminth
[a] Score constructed from five variables. Range = 0 – 5. Used as continuous
[b] Score constructed from four variables. Range = 0 – 5. Used as continuous
[c] Score constructed from 10 variables. Range = 0 – 10. Used as dichotomous. Cut-off = 6.0

An asset-based approach was used to establish the socio-economic status (SES) of the studied children (Raso et al., 2005). As shown in Table 9, the possession of five assets was used to produce the SES score. For electricity, TV and refrigerator, a value of 1 or 0 was assigned whether the family possessed or not the asset, respectively. For type of floor, a value of 0 was assigned if the house had only earthen floor; cement/mix floors or tile/mix floors were valued as 0.5 and 1, respectively. For type of sanitary facility, a value of 0 was assigned if the house lacked sanitary facility; latrine or toilet were valued as 0.5 and 1, respectively. LOWESS plots of the data were used to assess nonlinear relationship with the outcomes.

Table 9. Assets used for SES score calculation for the studied children (n = 317)

Variable	Category	Value
Electricity	No	0
	Yes	1
Refrigerator	No	0
	Yes	1
TV	No	0
	Yes	1
Type of floor [a]	Earthen only	0
	Cement or mix	0.5
	Tile or mix	1
Sanitary facility	None	0
	Latrine	0.5
	Flush toilet	1

SES: socio-economic status
[a] Three children did not recall type of floor at home

Awareness of STH was assessed based on four criteria for a maximum score of five points (Table 10). During the interview, children were asked if they were familiar with intestinal worms and if they thought they could acquire STH infections (risk perception). These questions had a dichotomous (yes / no) answer format with assigned values of 1 = yes and 0 = no. Additionally, children's knowledge of STH transmission and prevention was assessed using two open-ended questions with a value of 1.5 each. Rationale for adding weight to these questions was that knowledge of STH transmission and prevention reflect higher level of understanding. Children providing at least one correct answer to these questions received the respective full marks. LOWESS plots of the data were used to assess nonlinear relationship with the outcomes.

Table 10. Criteria used for calculation of STH awareness score for the studied children (*n* = 320)

Variable	Category	Value
Knowledge about STH	No	0
	Yes	1
Risk perception of STH infection	No	0
	Yes	1
Knowledge on STH transmission	No correct answer	0
	At least one correct answer	1.5
Knowledge on STH prevention	No correct answer	0
	At least one correct answer	1.5

STH: soil-transmitted helminth

School's hygienic conditions were assessed through visual inspection. A total of 10 criteria were used to calculate the school hygienic score (Table 11). For criteria with yes or no answer a value of 1 or 0 were assigned, respectively. For criteria with three possible outcomes, a value of 0.5 was assigned to the intermediate outcome. Once the score was calculated, the variable was dichotomized using an arbitrary cut-off value of 6.0; schools scoring below 6 were categorized as with low hygienic level.

Table 11. Criteria used for calculation of school hygienic score for the seven participant schools

Variable	**Category**	**Value**
Water was available during class time	No	0
	Yes	1
Sink with water was available for handwashing	No sink	0
	Only sink but no water	0.5
	Sink and water	1
Soap was available for handwashing	No	0
	Yes	1
Type of sanitary facility	None	0
	Latrine	0.5
	Flush toilet	1
Sanitary facility were functional	No	0
	Yes	1
Latrine / toilet were clean	No	0
	Yes	1
Toilet paper was available	No	0
	Yes	1
Floor in sanitary facility was clean	No	0
	Yes	1
Odors were controlled	No	0
	Yes	1
Surrounding areas were clean	No	0
	Yes	1

CHAPTER 4: RESULTS

4.1: Study participation

Seven of the nine visited schools were enrolled in the study (Figure 12). These schools belonged to the following communities: Colonia de Poncaya, Las Lomas de Poncaya, Las Parcelas, Corosito de Poncaya, El Cerro del Vigía, El Hormiguero, and Campamento Viejo. The two remaining schools (Santa Clara, n = 26 and Los Lirios, n = 19) were excluded due to recent deworming treatment and time-constraints to complete questionnaires, respectively. However, children from Los Lirios were examined for STH and treatment provided if needed. In total, 400 children enrolled in grades 3 to 5 were eligible to participate in the study. The parents of 368 (92%) children provided written informed consent for their children's participation and almost all of these children (357, 97%) assented to be enrolled. After enrolment, 37 participants were dropped from the study due to insufficient or no stool sample (n = 20), or unreliable Kato-Katz results that could not be repeated (n = 17). The final study sample consisted of 320 children of which, three children did not recall the type of floor at home and another 10 could not remember receiving previous deworming treatment.

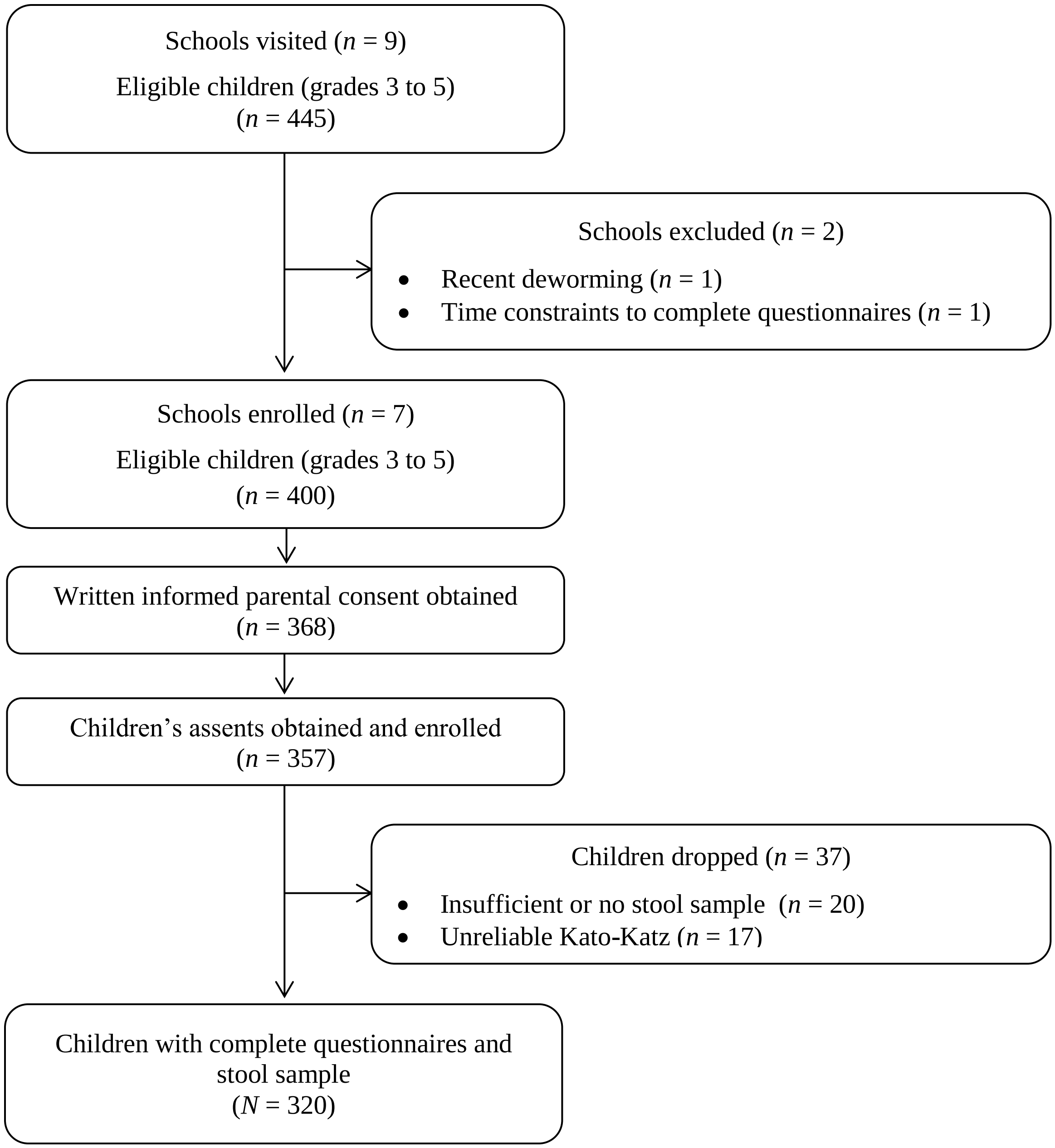


Figure 12. Flow chart detailing the study participation of children enrolled in seven rural schools of the Department of Olancho, Honduras, 2011.

4.2: Characteristics of the study population

The characteristics and parasitological findings of the study sample are presented in Table 12. Participating children were aged 7-14 years (mean 9.76 ± 1.4) and 154 (48%) of them were girls. About 37% of the children reported living in houses with partial or complete earthen floor, 49.8% had electricity and 87.5% had sanitary facilities available, either latrine or flush toilet. Habitual or occasional open defecation was reported by 28.4% of the children. As for STH history, 58.1% of the children reported having expelled worms in their lifetime and 88.7% recalled receiving deworming treatment sometime in the past but not recently. Approximately 20% of the children described doing outdoor chores on a regular basis and 50.9% mentioned walking barefoot when going outdoors. Most of the children (89.7%) reported the habit of washing hands regularly.

The majority of children (70%) scored low in the STH awareness scale (Table 12). Only 22.5% of them demonstrated good understanding of STH transmission / prevention (these children mentioned correct measures for preventing intestinal parasites). Conversely, the vast majority of children (96.9%) did not considered themselves at risk of acquiring STH infections.

Table 12. Characteristics and parasitological findings of the study sample (*N* = 320)

Characteristics	***n* (%)**
Age	9.76 (SD 1.4)
Girls	154 (48.1%)
Household conditions	
Earthen floor (complete or partial) (*n* = 317)[a]	118 (37.2%)
Electricity service	158 (49.8%)
Sanitary facility available	280 (87.5%)
Access to piped water	276 (86.3%)
Practices and STH history	
Habitual or occasional open defecation	91 (28.4%)
Reported regular handwashing	287 (89.7%)
Walked outdoor without shoes	163 (50.9%)
Performed chores outdoor	62 (19.4%)
Reported having passed worms in the past	186 (58.1%)
Recalled previous deworming (*n* = 310)[b]	275 (88.7%)
Socio-economic status (*n* = 317)[c]	
Poorest	94 (29.7%)
Very poor	48 (15.1%)
Poor	55 (17.4%)
Less poor	57 (18.0%)
Least poor	63 (19.9%)
Awareness and knowledge about STH	
Lowest awareness	89 (27.8%)
Lower awareness	137 (42.8%)
Higher awareness	22 (6.9%)
Highest awareness	72 (22.5%)
School hygienic conditions[d]	
Higher level	135 (42.2%)
School deworming regimen	
None or once a year	246 (76.9%)
Twice a year	74 (23.1%)
Parasitic profile	
Overall prevalence of STH infections	232 (72.5%)
Ascaris lumbricoides	97 (30.3%)
Trichuris trichiura	214 (66.9%)
Hookworms	51 (15.9%)
Polyparasitic infections (*n* = 232)	103 (44.4%)
Moderate-to-heavy infections by *Ascaris lumbricoides* (*n* = 97)	58 (59.8%)
Moderate-to-heavy infections by *Trichuris trichiura* (*n* = 214)	57 (26.6%)
Moderate-to-heavy infections by hookworms (*n* = 51)	3 (5.9%)

STH: soil-transmitted helminth
[a] Three children did not recall type of floor at home
[b] Ten children did not recall receiving previous deworming treatment
[c] Three missing cases. Socio-economic status score calculated only for 317 children
[d] School hygienic conditions scored 0-10. Schools which obtained scores higher than six were considered having higher level of hygiene compared to the rest

As explained in the methodology chapter, the possession of family assets was used to ascertain the socio-economic status (SES) score of the interviewed children. The SES score was calculated only for 317 participants since three children did not recall the type of floor at home, one of the variables used in SES calculation. Table 13 displays the SES score distribution by quintiles and it shows that wealth inequalities existed in the study population. This assessment shows that possession of electricity, refrigerators and flush toilets were important determinants of wealth. For instance, whereas almost 90% of the children in the highest SES quintile had a flush toilet at home, only 25% in the lowest quintile had such asset. As a compound score, SES showed a normal distribution in the study population with no significant skewness and slight negative kurtosis.

Table 13. Distribution of household assets among the study population by socio-economic status quintiles

Asset	**Having asset**	**SES quintiles (%)[a]**				
		Poorest	**Very poor**	**Poor**	**Less poor**	**Least poor**
	***n* (%)**	***n* = 94**	***n* = 48**	***n* = 55**	***n* = 57**	***n* = 63**
Electricity	158 (49.8)	0.0	8.3	65.5	96.5	100.0
Refrigerator	116 (36.6)	0.0	0.0	14.5	78.9	100.0
TV	151 (47.6)	1.1	2.1	60.0	93.0	100.0
Type of floor						
Earthen only	92 (29.0)	72.3	8.3	16.4	19.3	0.0
Cement or mix	171 (53.9)	26.6	83.4	63.6	68.4	50.8
Tile or mix	54 (17.0)	1.1	8.3	20.0	12.3	49.2
Sanitary facility						
None	40 (12.6)	33.0	2.1	10.9	3.5	0.0
Latrine	112 (35.3)	41.5	16.6	45.5	57.9	11.1
Flush toilet	165 (52.1)	25.5	81.3	43.6	38.6	88.9

SES: socio-economic status score

[a] Three missing cases, *n* = 317

Five of the seven participating schools had ongoing deworming programs, either twice a year (two schools) or once a year (three schools). They all used single-oral dose of 400 mg albendazole. School principals reported that the frequency of deworming depended on the availability of deworming medication at the school. Only 23% of the studied children had received deworming treatment at school twice a year (Table 12).

The schools' hygienic conditions were evaluated as described in the methodology chapter and the results are summarized in Table 14. Three schools obtained scores higher than the set cut-off value (6.0) and therefore, considered having higher level of hygiene compared to the rest.

About 42% of the studied children were attending schools with higher level of hygiene (Table 12). At the time of the inspection, only one school had soap available for handwashing and another one had toilet paper. According to principals, soap and toilet paper were rarely available in the school. Most schools had functional sanitary facilities, although they struggled with waste disposal in surrounding areas and odour control. Garbage was present in surrounding areas in six of the schools visited and only one school had a good control of odours from the sanitary facilities.

Table 14. School hygienic scores and criteria used for calculation of school hygienic scores for the seven participating schools

Code of school	Criteria[a]: Water during class time	Sink with water for handwashing [b]	Soap for handwashing	Latrine or toilet [c]	Sanitary facilities functional	Latrine / toilet clean	Toilet paper	Clean floor	Odours controlled	Clean surroundings areas	Hygienic score	Hygienic level
1	1	0	0	1	1	1	0	1	0	0	5.0	Low
2	0	0	0	0.5	0	0	0	0	0	0	0.5	Low
3	1	1	0	0.5	1	1	0	0	0	0	4.5	Low
4	0	0	0	0.5	1	1	0	0	0	0	2.5	Low
5	1	1	0	1	1	1	1	0	1	0	7.0	Higher
6	1	0.5	0	1	1	1	0	0	1	1	6.5	Higher
7	1	1	1	1	1	1	0	1	0	0	7.0	Higher

[a] Every criterion received a weight of 1 unless otherwise stated

[b] Sink and water = 1; only sink but no water available = 0.5

[c] Toilet = 1; latrine = 0.5

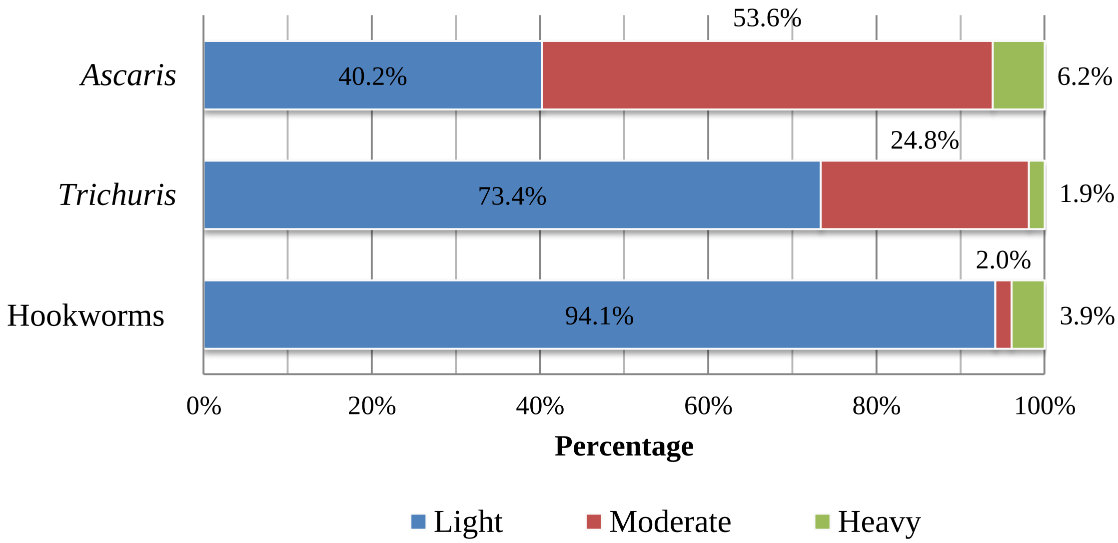
4.3: Parasitic profile of the studied children

4.3a: Prevalence of STH infections

A total of 232 of 320 children studied were infected with one or more intestinal helminths, for an overall point STH prevalence of 72.5% (95% CI = 67.3 - 77.3). Specifically, the prevalences for *A. lumbricoides*, *T. trichiura* and hookworms were 30.3% (95% CI = 25.3 - 35.7), 66.9% (95% CI = 61.4 - 72.0) and 15.9% (95% CI = 12.1 – 20.4), respectively (Table 12).

4.3b: Intensity of STH infections

As described before, based on the number of eggs per gram (epg) of stool, infection intensities were classified according to WHO criteria. As depicted in Figure 13, 40.2% of *Ascaris* infections were light whereas such intensity represented the majority of *T. trichiura* and hookworm infections (73.4% and 94.1%, respectively). More than half (53.6%) of ascariasis were of moderate intensity while only 24.8% of trichuriasis and 2% of hookworm infections accounted for such intensity.



Ascaris	1-4,999 epg	5,000-49,999 epg	≥ 50,000 epg
Trichuris	1-999 epg	1,000-9,999 epg	≥ 10,000 epg
Hookworms	1-1,999 epg	2,000-3,999 epg	≥ 4,000 epg

Figure 13. Intensity of STH infections among study population.

Heavy-intensity infections were uncommon, representing 6.2%, 1.9% and 3.9% of the cases of ascariasis, trichuriasis and hookworm infections, respectively. Overall, over one-third of all infections (84 of 232, 36.2%) were moderate-to-heavy.

4.3c: Polyparasitism

As shown in Table 15, of 232 infected children, 103 (44.4%) were polyparasitized. Of the latter, 27 (26.2%) harboured triple infections. *T. trichiura* prevailed across all combinations and it was found more frequently associated with *A. lumbricoides* than with hookworms.

Table 15. Proportion of cases with monoparasitism or polyparasitism among 232 infected children.

Type of infection	**Nº species**	**Species associated**	**Cases (%)**
Monoparasitism	1	*A. lumbricoides*	9/129 (7.0)
n = 129 (55.6%)		*T. trichiura*	113/129 (87.6)
		Hookworms	7/129 (5.4)
Polyparasitism	2	**Total two-species infections**	**76/103 (73.8)**
n = 103 (44.4%)		*T. trichiura* & *A. lumbricoides*	59/76 (77.6)
		T. trichiura & hookworms	15/76 (19.7)
		A. lumbricoides & hookworms	2/76 (2.6)
	3	**Total three-species infections**	**27/103 (26.2)**

STH: soil-transmitted helminth

4.4: Risk factors for STH infections

As explained earlier, 15 variables were chosen as potential risk factors and tested for associations with STH infection and intensity of infection in our study population. Among these variables, the two referring to SES and STH awareness were constructed as described in the methodology chapter and expressed as numerical scores, both ranging from 1 to 5. After examination of LOWESS plots, both variables showed linear relationship with the outcomes. For this reason, when running the logistic models, these variables were kept as continuous variables. The following sections describe the results obtained from these models.

4.4a: Risk factors and species-specific prevalence

After applying backward elimination, only variables with *p*-values ≤ 0.2 remained in the final regression models. Estimated OR from multivariable logistic models of species-specific prevalence are shown in Table 16. Within-school clustering was controlled using generalized estimating equations (GEE) approach.

Gender was only found associated with hookworm infection. Boys had twice the odds of being infected by this parasite (OR = 2.33, 95% CI = 1.23 – 4.41, *p* = 0.009). Age on the other hand, was not significantly associated with STH prevalences.

Socio-economic status (SES) had a protective effect against *Ascaris* (OR = 0.81, 95% CI = 0.67 - 0.98, *p* = 0.031) and *Trichuris* (OR = 0.78, 95% CI = 0.65 - 0.94, *p* = 0.007). Approximately, a decrease of 20% in the odds of being infected with any of these two parasites was observed per one (of five possible) units increase in the calculated SES score. Lacking any type of sanitary facility at home was a significant risk factor for hookworm infection (OR = 3.23, 95% CI = 1.38 - 7.56, *p* = 0.007). Hand washing did not show a significant association with the prevalence of any of the three parasites. However, children practicing occasional handwashing as opposed to regular handwashing had about three times more odds of harbouring infections by *Ascaris* and *Trichuris;* although statistical significance was only marginal (*p* = 0.051 and *p* = 0.064, respectively). Children who recalled receiving deworming treatment had 64% reduced odds of being infected by *Ascaris*, and this protective effect was statistically significant (OR = 0.36, 95% CI = 0.14 - 0.94, p = 0.036). The level of STH awareness played a significantly protective role against hookworms (OR = 0.72, 95% CI = 0.52 – 0.99, p = 0.040) but not against parasitoses by the other two species.

School deworming regimen was significantly inversely associated with *Ascaris* and hookworm infections. Children attending schools with absent or once-a-year deworming schedule had about 10 times the odds of being infected by *Ascaris* (OR = 10.32, 95% CI = 4.37 - 24.37, $p < 0.001$) and three times the odds of harbouring hookworm infections (OR = 2.92, 95% CI = 1.09 - 7.84, p = 0.033). In contrast, school deworming schedule was only marginally significantly associated with *Trichuris* infections (OR = 2.05, 95% CI = 0.95 - 4.42, p = 0.068).

School hygienic conditions were inversely associated with the prevalence of *Ascaris* and *Trichuris* infections. Children attending schools with low level of hygiene had almost 15 times greater odds of having ascariasis (OR = 14.84, 95% CI = 7.29 - 30.18, $p < 0.001$) and about seven times more odds of having trichuriasis (OR = 7.22, 95% CI = 3.66 - 14.22, $p < 0.001$).

Table 16. Multivariable logistic models of STH species prevalence using Generalized Estimating Equations (GEE) to account for within-school clustering.

Variables	***A. lumbricoides*** **n = 307** OR (95% CI)	*p*-value [a]	***T. trichiura*** **n = 317** OR (95% CI)	*p*-value [a]	**Hookworms** **n = 320** OR (95% CI)	*p*-value [a]
Age [b]	0.84 (0.67-1.05)	0.117	1.15 (0.92-1.43)	0.221	1.21 (0.96-1.54)	0.106
Sex						
Girls	1.00		--		1.00	
Boys	1.66 (0.91-3.03)	0.101	--		2.33 (1.23-4.41)	0.009
SES [c]	0.81 (0.67-0.98)	0.031	0.78 (0.65-0.94)	0.007	--	
STH awareness [d]	--		--		0.72 (0.52-0.99)	0.040
Handwashing						
Regularly	1.00		1.00		--	
Occasionally	2.62 (0.99-6.91)	0.051	2.72 (0.95-7.82)	0.064	--	
Sanitary facility						
Latrine or toilet	--		--		1.00	
None	--		--		3.23 (1.38-7.56)	0.007
Recalled deworming [e]						
No	1.00		--		--	
Yes	0.36 (0.14-0.94)	0.036	--		--	
School deworming						
Twice a year	1.00		1.00		1.00	
None/once a year	10.32 (4.37-24.37)	<0.001	2.05 (0.95-4.42)	0.068	2.92 (1.09-7.84)	0.033
School hygiene						
Higher level	1.00		1.00		--	
Low level	14.84 (7.29-30.18)	<0.001	7.22 (3.66-14.22)	<0.001	--	

OR: odds ratio; CI: confidence interval; STH: soil-transmitted helminth; SES: Socio-economic status
[a] *p*-value from likelihood ratio test
[b] Age as continuous variable in years
[c] SES score calculated as described in methodology section. Range = 0 – 5. Three missing data (*n* = 317)
[d] STH Awareness score calculated as described in methodology section. Range = 0 – 5
[e] Ten children did not recall receiving previous deworming treatment
(--) Variable not included in the final model

4.4b: Risk factors and polyparasitism

Estimated odds ratios (OR) from multivariable GEE logistic model of polyparasitism are shown in Table 17. Few variables were found significantly associated with multiple-parasite infections. Polyparasitism had twice the odds of occurring in boys (OR = 2.12, 95% CI = 1.20 - 3.76, p = 0.010).

Children's level of STH awareness seemed to have a protective effect against polyparasitism. A 25% decrease in the odds of being polyparasitized was observed per one (of five possible) units of awareness increase (OR = 0.75, 95% CI = 0.57 - 0.98, p = 0.035). Similarly, recalling previous deworming was associated with a 45% reduction in cases of polyparasitism, although such association did not reach statistical significance (p = 0.164). Conversely, open defecation increased the risk of polyparasitism. Children practicing habitual or occasional open defecation had almost twice the odds of being polyparasitized. However, this association was only marginally significant (OR = 1.72, 95% CI = 0.95 - 3.13, p = 0.073).

School environment also played a role in polyparasitism. School hygienic conditions and deworming schedule were significantly associated with polyparasitism. Children in schools with low hygienic conditions had nine times increased odds of being polyparasitized (OR = 9.22, 95% CI = 4.72 - 17.98, $p < 0.001$). Similarly, the odds of polyparasitism significantly increased in children attending schools with absent or once-a-year deworming schedule (OR = 11.36, 95% CI = 4.96 - 26.01, $p < 0.001$).

Table 17. Multivariable logistic models of polyparasitism using Generalized Estimating Equations (GEE) to account for within-school clustering.

Variables	**Polyparasitism (n = 310) OR (95% CI)**	***p*-value [a]**
STH awareness [b]	0.75 (0.57-0.98)	0.035
Sex		
Girls	1.00	
Boys	2.12 (1.20-3.76)	0.010
Open defecation		
No	1.00	
Yes	1.72 (0.95-3.13)	0.073
Recalled previous deworming [c]		
No	1.00	
Yes	0.55 (0.24-1.27)	0.164
School deworming		
Twice / year	1.00	
None-once / year	11.36 (4.96-26.01)	<0.001
School hygiene		
Higher level	1.00	
Low level	9.22 (4.72-17.98)	<0.001

OR: odds ratio; CI: confidence interval; STH: soil-transmitted helminth

[a] *p*-value from likelihood ratio test

[b] STH Awareness score calculated as described in methodology section

[c] Ten children did not recall receiving previous deworming treatment

4.4c: Risk factors and species-specific infection intensity

Estimated OR from multivariable GEE logistic models of STH infection intensity are shown in Table 18. Since the vast majority (94.1%) of hookworm infections were light, models only were constructed for *Ascaris* and *Trichuris*.

Gender was not significantly associated with infection intensity; however, even when not reaching statistical significance, moderate-to-heavy *Ascaris* infections had almost twice the odds of occurring in boys ($p = 0.059$).

Having earthen floor at home was identified as a significant risk factor for moderate-to-heavy trichuriasis (OR = 2.06, 95% CI = 1.07 - 3.96, $p = 0.030$). Walking barefoot outdoors was associated with moderate-to-heavy trichuriasis (OR = 2.51, 95% CI = 1.30 - 4.84, $p = 0.006$) but this practice was only marginally significantly associated with ascariasis (OR = 1.75, 95% CI = 0.91 - 3.38, $p = 0.096$).

As in the case of infection prevalence, deworming regimen played a relevant role in intensity of infection. Children in schools with absent or once-a-year deworming schedule had significantly increased odds of having moderate-to-heavy ascariasis (OR = 7.92, 95% CI = 3.14 - 19.96, $p < 0.001$) and trichuriasis (OR = 8.57, 95% CI = 2.86 - 25.64, $p < 0.001$). Additionally, low hygienic conditions in the schools were significantly associated with higher intensity infections with *Ascaris* (OR = 13.95, 95% CI = 5.61 - 34.69, $p < 0.001$) and *Trichuris* (OR = 2.95. 95% CI = 1.45 - 6.00, $p = 0.003$).

Table 18. Multivariable logistic models of infection intensity of *Ascaris* and *Trichuris* using Generalized Estimating Equations (GEE) to account for within-school clustering.

Variables	***A. lumbricoides*** **Moderate-to-heavy [a]** ***n*** **= 320** **OR (95% CI)**	***p*-value [b]**	***T. trichiura*** **Moderate-to-heavy [a]** ***n*** **= 317** **OR (95% CI)**	***p*-value [b]**
Sex				
Girls	1.00		--	
Boys	1.87 (0.98-3.59)	0.059	--	
Shoes when outdoors				
Wear shoes	1.00		1.00	
Barefoot	1.75 (0.91-3.38)	0.096	2.51 (1.30-4.84)	0.006
Earthen floor [c]				
No	--		1.00	
Yes	--		2.06 (1.07-3.96)	0.030
School deworming				
Twice / year	1.00		1.00	
None-once / year	7.92 (3.14-19.96)	<0.001	8.57 (2.86-25.64)	<0.001
School hygiene				
Higher level	1.00		1.00	
Low level	13.95 (5.61-34.69)	<0.001	2.95 (1.45-6.00)	0.003

OR: odds ratio; CI: confidence interval

[a] Moderate-to-heavy vs. non-infected-to-light

[b] *p*-value from likelihood ratio test

[c] Three children did not recall type of floor at home

(--) Variable not included in the final model

CHAPTER 5: DISCUSSION

Despite the ubiquitous nature of childhood soil-transmitted helminthiases in Honduras (PAHO, 2009), scientific research examining risk factors involved in the transmission of these infections is scarce. Through the extensive search of the peer-reviewed national and international published literature done for this thesis, only seven Honduran community-based studies assessing STH prevalence in children were found; none of which investigated risk factors (Kaminsky, 1997; Kaminsky et al., 2000; Kaminsky and Retes, 2000; Zepeda, 1972; Zepeda and Barahona, 1970a; Zepeda and Barahona, 1970b, 1971). The literature search identified one additional study investigating various risk factors associated with the prevalence of *Ascaris* and *Trichuris* but the focus of the study was the general population, not children (Smith et al., 2001). Hence, to our knowledge, the present study is the first to undertake a comprehensive investigation of the biological, behavioral, socio-economic, and environmental factors playing a role in the prevalence and intensity of STH infections in Honduran children.

5.1: Prevalence of soil-transmitted helminths

We found a high prevalence of STH infections in the studied children. High prevalence of STH was also found by the Honduran Ministry of Health (MoH), in a sample similar to that of the present study, both in composition and size (*i.e.*, 230 school children 9-11 years of age residing in three rural communities in Olancho). That study was part of the 2011 national survey done by the Honduran Ministry of Health (MoH). Differences between the two studies are evident. Our findings show an overall STH prevalence of 72.5% while the MoH reports 39%. Likewise, our study shows specific prevalences for

Ascaris, *Trichuris* and hookworms of 30.3%, 66.9% and 15.9%, respectively. In contrast, for the same parasites, the MoH reported prevalences of 17.7%, 30.6% and 0%, respectively (Ministry of Health Honduras, 2011).

Interpretation of these marked differences must be done with caution, as both the MoH survey and the present study are cross-sectional investigations with inherent design biases. However, it is worth noting that the studied communities while not the same, are located within the same ecological area (Honduras, 2010). They are also ranked very closely in terms of human development (UNDP, 2012). Hence, it is possible that the underlying cause of prevalence disparities lies on the uneven distribution of deworming treatment among Honduran communities. As presented in the Results section, our study detected that despite close geographical proximity, deworming activities were dissimilar among participating schools.

The prevalence disparity between the two studies could be also related to real differences in transmission and that our studied communities are hyperendemic foci or "hotspots", where intense transmission of STH takes place (Schneider et al., 2011). This explanation is plausible since it has been observed that seemingly homogenous communities can in fact be very heterogeneous when it comes to transmission intensity (Brooker et al., 2006; Lustigman et al., 2012).

Finally, a further explanation of these differences could lie on methodological issues. Small-scale research studies, being able to deploy more resources than national surveys, might be better equipped to control for error. Parasitological findings relying on Kato-Katz, for example, can be subject to great variability depending on operational factors (Booth et al., 2003; Knopp et al., 2008; Santos et al., 2005; WHO, 1994). Our previous experience showed that extended Kato-Katz clarification time reduced hookworm detection by 50% (Gabrie et al., 2012); therefore, quality control is integral part of our laboratory procedures. We are not aware of laboratory difficulties occurring with the MoH surveys, but diagnostic reliability is worth investigating. Future surveys would benefit from including quality control protocols in their laboratory methodology.

Determining prevalence with precision is essential to public health policy and practice. According to WHO (2012), any area with a prevalence of STH ≥50% among school-age children is considered a high-risk area and merits a bi-annual deworming schedule (WHO, 2012). Our data support this re-treatment schedule whereas official data suggest that once a year would be sufficient.

5.1a: STH species prevalence

In the present study trichuriasis was the most predominant of the three parasitoses investigated (66.9%), with ascariasis and hookworm infections ranking in distant second and third places (30.3% and 16%, respectively). Our findings support recent reports showing that in Latin America, the estimated number of *T. trichiura* infections surpasses those of *Ascaris* (100 million versus 84 million, respectively) (Hotez et al., 2008). Globally, however, it seems that *A. lumbricoides* remains the most common of the three STH (Brooker and Utzinger, 2007; de Silva et al., 2003; Utzinger, 2012). In Honduras, data showing predominance of either parasite have been produced over the years. *T. trichiura* was the predominant STH in previous research studies (Sanchez et al., 1997; Sosa, 2007) and the three national surveys (Ministry of Health Honduras, 2003, 2006, 2011). Similar findings have also been recently reported in Nicaragua (Rosewell et al.,

2010) and El Salvador (Corrales et al., 2006). In contrast, the majority of older studies in Honduras (Kaminsky et al., 2000; Smith et al., 2001; Zepeda, 1972; Zepeda and Barahona, 1970a; Zepeda and Barahona, 1970b, 1971), Guatemala (Anderson et al., 1993; Cook et al., 2009; Jensen et al., 2009; Sorensen et al., 2011; Watkins and Pollitt, 1996) and Nicaragua (Oberhelman et al., 1998; Tellez et al., 1997), reported a higher prevalence for *A. lumbricoides* than for *T. trichiura.*

The reasons for the current predominance of *T. trichiura* in the Honduras have yet to be elucidated but three plausible factors can be at play. Firstly, the laboratory methodology used for parasite detection has changed. More recent studies have used Kato-Katz, a method with higher sensitivity for light infections than the direct wet mount traditionally used prior the 1990's (WHO, 2002). Secondly, the widespread implementation of preventive chemotherapy (PC) could be driving the species shift. As explained previously, deworming campaigns are primarily based on single-dose albendazole treatments, and while this drug is highly efficacious against *Ascaris* and hookworms, it only shows moderate-to-low efficacy against *Trichuris* (Keiser and Utzinger, 2008; Vercruysse et al., 2011b). Finally, anthelminthic resistance could be emerging. Recent work by Diawara and collaborators (2013) strongly suggests that the mechanism underlying this selective pressure is that albendazole selectively eliminates worms carrying susceptible-type alleles (β-tubulin gene at position 200) allowing worms with the resistance-type alleles to survive treatment. Further evidence has been found in studies following populations after treatment. Significant increase of homozygous resistance-type parasites have been observed in Haiti and in Kenya (Diawara et al., 2013a).

The emergence of anthelminthic drug resistance in human parasites is subject to increasing attention. Advocates emphasize the need to continue monitoring the efficacy of anthelminthic drugs and underscore the importance of elucidating the genetic and molecular basis of anthelmintic resistance (Montresor, 2011; Vercruysse et al., 2011a). In Honduras, the epidemiological and health implications of *T. trichiura* predominance need to be properly determined and addressed.

The unexpectedly high prevalence of hookworm infections (15.9%) observed in the present study is worth highlighting. The studied communities did not have the optimal environmental conditions (*i.e.*, sandy/loamy soils and rainfall of 750-1250 mm in the warm months) described for hookworm transmission (Bogitsh et al., 2012; Nelson and Masters, 2007). On the contrary, these communities had clay-rich soils and low pluvial precipitation -about 200 mm in the wettest month (http://www.weather-and-climate.com; accessed: July 23th, 2013). It would be valuable to investigate the existence of microenvironments that sustains hookworm eggs' maturation, their evolution into infective stages and larvae survival, within an ecological area with apparent adverse conditions to hookworm transmission. It would also be worthwhile exploring the possibility of zoonotic transmission (Conlan et al., 2012; Mahdy et al., 2012; Ngui et al., 2012) or other alternative hookworm transmission mechanisms that have been described in the literature (*e.g.*, oral transmission) (Bogitsh et al., 2012; Roberts and Janovy, 2009b; Tiwari et al., 2004; Yu et al., 1995).

The high prevalence of all parasite species underscores the need for regular deworming and monitoring in the studied communities.

5.2: STH infection intensity

Our study shows that 59.8%, 26.6%, and 5.9% of infections with *A. lumbricoides*, *T. trichiura,* and hookworm, respectively, were of moderate or high intensity. The recent strategic plan put forward by the WHO establishes that STH are a public health problem when "the prevalence of STH infection of moderate and high intensity among school-children is over 1%" (WHO, 2012 page 20). Evidently, STH are a public health problem in the studied communities but an assessment of childhood morbidity caused by these infections is yet to be done anywhere in the Honduran territory.

The global goal of eliminating STH as a public health problem entails reducing morbidity from STH to an acceptable level. This means that light-intensity infections assumed to carry negligible morbidity (Crompton et al., 2003; Moreau and Chauvin, 2010) will be given low priority (Ezeamama et al., 2005; Pullan and Brooker, 2008). However, several studies have demonstrated that concomitant infections, even if of light intensity, may have an additive or synergistic detrimental effect, especially in children (Ezeamama et al., 2005; Mupfasoni et al., 2009; Pullan and Brooker, 2008). In malnourished children, light STH infections may contribute to growth deficits (Crompton and Nesheim, 2002; Stephenson et al., 2000). It is therefore important to establish the actual health burden of STH infections of any intensity in Honduran children. Along with expanding deworming coverage and improved sanitation to interrupt transmission, research into the health impact of STH in children is also necessary in Honduras.

5.3: Polyparasitism

In the present study, a high proportion (44.4%) of infected children harboured multiple parasites (two or three helminths). Out of these, almost three quarters (73.8%) were infected with two STH species, most commonly with both *A. lumbricoides* and *T. trichiura*. Since these two parasites have a common transmission pattern (Bogitsh et al., 2012; Hotez, 2008a), this finding was predictable and agrees with previous studies conducted in El Salvador (Corrales et al., 2006), Guatemala (Sorensen et al., 2011), Colombia (Agudelo-Lopez et al., 2008), India (Anbumani and Mallika, 2011), Philippines (Belizario et al., 2003) and Ethiopia (Alemu et al., 2011). In highly endemic countries, polyparasitism is the usual pattern due to the coincidence in time and space of multiple host-parasite relationships (Lustigman et al., 2012; Tchuem Tchuente et al., 2003).

As mentioned previously, the health impact of multiple parasite infections can be higher than the sum of single-species infections (Brooker et al., 2000; Ezeamama et al., 2008; Mupfasoni et al., 2009). It has been proposed that through immunomodulatory effects, polyparasitism may increase susceptibility to other infections such as malaria or HIV (Maizels and Yazdanbakhsh, 2003; Nacher, 2004). But scientific evidence in this regards is still inconclusive (Supali et al., 2010), so the importance of concurrent infections is yet to be determined at the policy level.

5.4: Risk factors for STH

Identifying risk factors involved in the transmission of STH is not without challenges. Social structural determinants such as poverty, lack of safe water and sanitation are recognized drivers for STH distribution (Brooker et al., 2006; Hotez et al., 2003; WHO, 2012). Yet, substantial differences in the level of endemicity have been observed within populations living in similar communities (Nishiura et al., 2002; Smith et al., 2001). This suggests the existence of unidentified factors that either predispose or protect against STH infection. Identifying such factors would enable researchers to suggest feasible interventions conducing to effective and sustainable STH control (Gazzinelli et al., 2012; Weaver et al., 2010). The present study identified several factors associated with STH prevalence and intensity in a sample of Honduran children living in seven rural communities, which at first glance appeared to be similar. A discussion of the factors investigated follows.

5.4a: Age

Age of the child was not identified as a significant risk factor for infection in the present study. However, as described below, we detected non-statistically significant trends related to age and infection that are worth discussing. This observation may be explained by the narrow age range (7 - 14 years) of the study sample and/or small sample size. Most studies involving school-age children present similar findings (Alemu et al., 2011; Ekpo et al., 2008; Hesham Al-Mekhlafi et al., 2008; Lello et al., 2013; Scolari et al., 2000; Sorensen et al., 2011; Standley et al., 2009; Steenhard et al., 2009; Yami et al., 2011).

Although not statistically significant, our analysis showed that as the age of children increased by one year, the odds for ascariasis were reduced by about 20%. This is consistent with the age-related pattern described for *A. lumbricoides* (Dold and Holland, 2011) which likely reflects acquisition of a combination of protective factors such as partial immunity and decrease exposure to the parasite. Reports from India, in 204 school children aged 5 - 9 years (Naish et al., 2004) and El Salvador, in 449 participants of all groups of age (Corrales et al., 2006), were able to identify this pattern. Conversely, we observed that as the age of children increased by one year, the odds of having trichuriasis were increased by 15%. Since the mode of infection and transmission mechanism for both *A. lumbricoides* and *T. trichiura* are similar, they are usually found causing concurrent infections, as described above. Our result is in agreement with the findings obtained in a recent study in Laos (Conlan et al., 2012) and differed with other studies asserting that trichuriasis is more common in younger children (Agudelo-Lopez et al., 2008; Bundy and Cooper, 1989).

In terms of hookworm infections, we observed that as the age of children increased by one year, the odds of being infected with these parasites increased by 20%. This finding corresponds with the pattern of infection described for hookworms (Nelson and Masters, 2007). A few studies in children with similar age range than the present study observed higher hookworm prevalence in older children (Alemayehu, 2008; Odiere et al., 2011; Soares Magalhaes et al., 2011; Steenhard et al., 2009).

Identifying whether parasitism peaks at pre-school age or throughout primary school years has important implications for control strategies. Since the majority of studies have focused on school-age children, more evidence exists for this age group.

Nevertheless, as exposure to STH starts earlier in life for children living in endemic countries (Gyorkos et al., 2011b), the current WHO recommendations call for including children of all ages in the deworming programs. A benefit to this approach is that deworming earlier would likely prevent detrimental effects of STH in "the first 1000 days", the most vulnerable in the life of a human being (WHO, 2013).

5.4b: Sex

Sex of the child (being a boy) was significantly associated with increased prevalence of hookworm infection. We also found that polyparasitism was significantly higher in boys than in girls. Studies on hookworm infections worldwide have consistently identified higher prevalence in males (Alemayehu, 2008; Gunawardena et al., 2011; Hohmann et al., 2001; Lello et al., 2013; Soares Magalhaes et al., 2011; Steenhard et al., 2009). To explain this phenomenon, scientist have cited differential exposure (Bundy, 1988) while others have proposed that physiological factors might play a crucial role (Poulin, 1996; Zuk and McKean, 1996). More recently, it has been argued that the root cause is probably due to a combination of both factors (Brooker et al., 2004; Hotez et al., 2004).

Studying physiological factors was beyond the scope of the present study but we had the opportunity to collect some information in regards to children's game playing habits that offer a glimpse to differential exposure. For example, at the interview, 84.3% of boys referred practicing "high-soil contact" games (*e.g.*, soccer, ball game, marbles, playing with soil/mud and stones, etc.) whereas only 26.6% of the girls practiced such type of games. Since we did not include this variable into our regression models, we lack the statistical analysis to relate this observation to prevalence data. This is nevertheless an interesting finding worth investigating, perhaps through qualitative research or, perhaps better, using a combination of qualitative and quantitative research (mixed method). Other infectious diseases such as schistosomiasis (Michelson, 1993) and tuberculosis (Uplekar et al., 1999) exhibit a distinctive prevalence in males which has been attributed to differential exposure rather than biological differences. On the other hand, recent experimental data have lent support to repeated epidemiological observations identifying a higher prevalence of human tapeworm infections in females due to hormonal factors (Nava-Castro et al., 2012). In regards to STH, lack of experimental studies prevents any argument either in favour or against the relationship of physiological factors to infection prevalence.

5.4c: Household conditions

About 40% of the studied children referred having either complete or partial earthen floor at home but this finding was not associated with STH prevalence. However, this type of floor showed an association with intensity of infection of *T. trichiura* whereby children reporting this household characteristic had twice the odds of harbouring moderate-to-heavy trichuriasis.

Household conditions may play an important role in STH transmission since the physical environment of the house and peridomestic area can facilitate parasites' transmission cycles (Cairncross et al., 1996). Transmission of *A. lumbricoides* and *T. trichiura* is thought to mainly occur in the private or domestic domain (household and peridomestic area) while several other infections, including hookworms, are believed to be transmitted in both the private and public domains (Olsen et al., 2001). Earthen floors pose the greatest opportunity for STH transmission than other type of floor since it can be potentially contaminated with feces and once this occurs, they are difficult to clean.

Earthen floors have been identified as risk factors for *A. lumbricoides* (Walker et al., 2011), *T. trichiura* (Corrales et al., 2006) and hookworm infections (Corrales et al., 2006; Soares Magalhaes et al., 2011).

Only 12.5% of the studied children stated not having an adequate option for fecal disposal at home. According to the Honduran government, in 2011 only 15.5% of households in rural Honduras were lacking sanitary facilities (http://www.ine.gob.hn/drupal/, accessed July 23th, 2013). Therefore, the information obtained from the children concurs with official information.

Despite the high prevalence of parasitism found among the children, our study did not find an association between the absence of a sanitary facility at home and infections by *T. trichiura* or *A. lumbricoides.* However, we did find such association for hookworm infections. Children living in households without sanitary facilities had three times greater odds of being infected with hookworm than children reporting access to a latrine or toilet at home. Similar observations have been reported in comparable studies in Guinea-Bissau (Steenhard et al., 2009), and Burkina-Faso, Ghana and Mali (Soares Magalhaes et al., 2011). Due to their mode of transmission, lack of sanitary facilities have been associated with STH infections in general (Gamboa, 2011; Gamboa et al., 2009; Hesham Al-Mekhlafi et al., 2008; Scolari et al., 2000). Repeated return to defecation sites exposes people to continued reinfection (Roberts and Janovy, 2009b). A comprehensive approach to STH control is then required, and in addition to mass deworming it should include household and environmental sanitation.

The source of water has been investigated as a potential risk factor for STH infections and some studies have associated the lack of a safe source of water with an increased risk for helminth infections (Alemayehu, 2008; Alemu et al., 2011; Ngui et al., 2011; Scolari et al., 2000; Steenhard et al., 2009; Walker et al., 2011). About 86% of the children in our study reported having piped water at their household but we could not demonstrate a significant association between lack of piped water and STH infections. Other researchers have failed to find this connection as well (Balen et al., 2011; Corrales et al., 2006; Hesham Al-Mekhlafi et al., 2008). In our case, the high proportion of households with piped water or water treatment practices (half of the households treated the water before drinking) may have prevented us from finding statistical significance. Nevertheless, it is important to recall that drinking contaminated water is not the chief mechanism of STH transmission. Soil fecal contamination is a mandatory step for STH eggs maturation and thus, inadvertent ingestion of contaminated soil containing infectious stages is the primary mechanism of transmission. Secondary contamination of drinking water carries important potential but it is not always identified as a risk factor in epidemiological studies.

5.4d: Socio-economic status

Socio-economic status (SES) of the studied children was identified as a risk factor for ascariasis and trichuriasis. Significantly lower odds of being infected by these parasites were found in children with higher SES score. Families with higher SES are expected to live in households with better sanitary conditions that may prevent the transmission of STH infections within the domestic domain, where *A. lumbricoides* and *T. trichiura* are mainly transmitted (Cairncross et al., 1996; Olsen et al., 2001). Our findings are in alignment with studies conducted worldwide that identified low SES as an important risk factor for STH infections (Balen et al., 2011; Conlan et al., 2012; Halpenny et al., 2013; Hohmann et al., 2001; Kounnavong et al., 2011; Sayasone et al., 2011; Steinmann et al., 2007). STH infections are rooted in poverty (Hotez et al., 2008; WHO, 2012). This is easy to grasp when looking at the geographical distribution of these parasites: they are disproportionally concentrated in low and middle income countries (WHO, 2010). Identifying endemic foci within already poor countries proves more challenging. Not all studies are able to demonstrate this association, though, as reliable measurement of SES in developing countries is extremely difficult (Nematian et al., 2004).

5.4e: STH awareness

As mentioned previously, very few children (22.5%) in this study were able to provide correct answers regarding STH transmission and prevention. Further, the vast majority (96.9%) of participants did not perceived being at risk for STH infections. Interestingly, STH awareness showed a significant protective effect against hookworm infections and polyparasitism in the studied children. Odds of being polyparasitized or harbouring hookworm infections were about 30% lower in children with higher awareness. Increasing the level of awareness has been proved to be effective in preventing STH infections (Fouamno Kamga et al., 2011) and accordingly, health education has become a major component of comprehensive STH control programs (Montresor et al., 2011; WHO, 2010). However, passive health education alone may not translate into behaviour change. Personal willingness and ability to practise behaviours that reduce the risk of infection is fundamental to the success of health education initiatives (Anantaphruti et al., 2008). Our study communities would benefit from improved health education at the school and family level. But tailoring health messages according to cultural and societal values and beliefs is a first and mandatory step for the success of educational programs (WHO, 2012). Identifying the types of health messages and the most effective media to convey them can be achieved through the integration of the health and education sectors with the participation of endemic communities.

5.4f: Open defecation

In contrast to the finding that only 12.5% of children referred lacking sanitary facilities at home, a higher proportion of them (about 30%) reported practicing either habitual or occasional open defecation (OD). A variety of explanations can be offered to address this discrepancy. Most importantly, information in regards to actual usage or functionality of these facilities at home was not collected from the research participants. Additionally, some children attended schools without such facilities and might have been forced to practice OD. In view of their personal nature, asking about defecation practices could pose accuracy issues in epidemiological studies. Research shows that participants tend to provide socially acceptable answers in these cases (Díaz et al., 2010) and it is possible that OD practices were even higher among the children. This could explain the fact that we did not find statistical significance even when OD is a confirmed risk factor for STH. Other studies in school children from Ethiopia (Alemayehu, 2008; Alemu et al., 2011) and Pakistan (Nishiura et al., 2002) could not demonstrate an association either.

Besides reliability issues, another potential explanation can be offered. In highly endemic communities, regardless of SES, household conditions or individual behaviour, people are widely exposed to environmental contamination. Such indiscriminate exposure has the potential to override other protective factors, especially for children.

5.4g: Handwashing

Even if the association was only marginally significant, it is important to highlight that in the present study children practicing only occasional handwashing had almost three times higher odds of being infected by *A. lumbricoides* and *T. trichiura* than their counterparts who reported regular handwashing. The lack of statistical significance is worth commenting as almost 90% of the children reported washing their hands regularly. This high reporting of handwashing seems consistent with the reported access to piped water in the household (86%) and may reflect children's true good hygiene habits when at home.

However, as discussed below, 60% of schools to which children were attending did not have water for handwashing and therefore, the reliability of handwashing data is put to doubt. Moreover, as indicated for OD above, questioning about handwashing habit may lead to overestimation of the practice as the research participant might have the desire to provide a socially acceptable response and to satisfy the interviewer (Borchgrevink et al., 2013; Díaz et al., 2010; Monk-Turner et al., 2005). More objective assessment of hygiene behaviours should be used to draw meaningful conclusions. Notwithstanding, the importance of handwashing should not be disregarded as it is a cost-effective and important hygiene measure in preventing the spread of infectious diseases (WHO, 2009) and has been proven effective in preventing intestinal helminths (Alemayehu, 2008; Alemu et al., 2011; Conlan et al., 2012; Fung and Cairncross, 2009; Hohmann et al., 2001).

5.4h: Wearing shoes outdoors

Half of the studied children reported walking outdoor without shoes. Walking barefoot has been identified as a risk factor for STH infections, especially by hookworms (Alemu et al., 2011; Bleakley, 2007). However, the work of Lello and colleagues (2013) demonstrated that walking barefoot can be also a risk factor for trichuriasis (Lello et al., 2013).

Interestingly, the results of the present study identified walking barefoot as a significant risk factor for *T. trichiura* intensity of infection, although not for the presence of infection *per se*. Higher infection intensities were found in children who reported walking outdoor barefoot. This finding might be explained in terms of an increased exposure to contaminated soil with embryonated eggs of the parasite. Children may have touched their contaminated feet and eggs transferred to their hands and eventually their mouths if hand hygiene was not optimal. The fact that we did not find this association for *A. lumbricoides* is puzzling as both parasites have similar mechanism of transmission. Even more striking was not finding a link between walking barefoot and hookworm infection (since hookworms are chiefly acquired through the feet when larva in the soil enters in contact with the feet and penetrates the skin).

5.4i: STH history

In endemic areas, helminth infections are usually accepted as a normal and unavoidable reality (Tanner et al., 2009). When asked about past infections, almost 60% of the studied children recalled having passed worms. Although this answer mostly referred to *A. lumbricoides* that, due to its size, is more visible than *T. trichiura* and hookworms, it highlights how common these infections are in the study communities. Upon fecal microscopic examination, 30% of the children were found infected with *A. lumbricoides*, which lends support to the children's recollection of infection.

Further evidence of the ubiquity of STH infections in the studied community is provided by the fact that, almost 90% of the children recalled previous deworming.
The effects of deworming activities were evident in the studied children. A history of previous deworming was significantly associated with a 64% reduction in the odds of having ascariasis. Considering that the current PC regime in Honduras (*i.e.*, single-dose albendazole or mebendazole) are very efficacious against *A. lumbricoides* but not against *T. trichiura* (Keiser and Utzinger, 2008; Vercruysse et al., 2011b), these results underscore the need for targeted interventions at the sub-national level, instead of a homogenized national approach.

5.4j: School environment

An interesting finding in this study was that hygienic conditions at schools played a major role in both transmission and intensity of *A. lumbricoides* and *T. trichiura,* and also in the polyparasitism observed. Schoolchildren spend a good part of the day (about 5 hrs.) at school; learning in the classrooms but also playing in recreational areas. This close interaction among pupils within the school environment may favour disease transmission. In addition inadequate hygienic conditions at the school may have promoted fecal contamination of playgrounds' soil with helminths' eggs from infected children (Bogitsh et al., 2012). Future research could investigate the degree of soil contamination in the schools as parasite's eggs persist in the external environment for long periods of time. As the PC program in Honduras reaches the desired national coverage, it would be important to address environmental reservoirs for infection.

The role of schools' hygiene conditions in children's lives was evident in the present study. As mentioned, handwashing is an important measure to interrupt infectious disease transmission but handwashing *per se* does not guarantee infection prevention if it is not properly done (*i.e.*, using soap and safe water) (Bartram and Cairncross, 2010; Snow et al., 2008; WHO, 2009). The fact that soap was not available in six of the seven schools enrolled in the study and that almost 60% of the schools did not have water available for handwashing suggest that more attention should be paid to the school environment and conditions.

Hygienic conditions at school did not play a role in hookworm infections. A plausible explanation for this funding stems from the fact that free hookworm larvae are less resistant to desiccation and radiation than the infective forms of *Ascaris* and *Trichuris* which are protected within thick-shelled eggs. As per researchers' observations, school's playgrounds and surrounding areas lacked vegetation and the soil was considerably dry and compact; conditions not at all favourable for hookworm transmission. In addition to this biological explanation, statistical power may have been insufficient to detect any association in this regard as the number of hookworm infections in the studied communities was far smaller than that of than trichuriasis and ascariasis.

5.4k: School deworming regimen

Schools' frequency of deworming also played an important role for STH transmission and intensity. Despite the relative proximity of the studied communities, deworming regimen at schools was inconsistent with 77% of the children having received no deworming treatment or being treated only once a year. These children had significantly higher odds of being infected by *A. lumbricoides* and hookworms, as well as of being polyparasitized. Similarly, they had eight time higher odds of harbouring moderate-to-heavy ascariasis and trichuriasis. The main goal of preventive chemotherapy (PC) is to reduce STH morbidity. However, for achieving this goal, the PC must be administered in the adequate frequency (Montresor et al., 2012). In highly endemic areas (≥ 50% prevalence) as the studied communities, regimen of single-dose albendazole / mebendazole twice or, if possible, three times a year are recommended by the WHO (WHO, 2006, 2012).

Interestingly, in the present study the frequency of deworming in the schools showed no effect in *T. trichiura* transmission. As mentioned previously, single-dose albendazole / mebendazole is less efficacious against this parasite (Adams et al., 2004; Keiser and Utzinger, 2008; Vercruysse et al., 2011b). It would seem that, contrary to what we observed for *A. lumbricoides* and hookworms, PC helped to reduce *T. trichiura's* worm load (intensity of infection) but had little effect on the parasite's transmission.

Our findings stress the need for Honduras to continue and sustain its deworming program. They also reveal the need to implement and monitor integrated STH control efforts (Kabatereine et al., 2010; Knopp et al., 2011b). Baseline studies measuring reinfection rates (Halpenny et al., 2013) and drug efficacy (Albonico et al., 2004), along with efforts to detect potential emergence of resistance to benzimidazoles (Diawara et al., 2013b; Vercruysse et al., 2011a) would better inform the STH control efforts in the country.

5.5: Study strengths and limitations

This study has some important strengths. Firstly, the cross-sectional design of the study was appropriate for meeting the research objectives. Secondly, a high participation rate was obtained. Thirdly, our sample was likely representative of the communities' school children as in Honduras 95% children attend primary school (United Nations, 2010). Fourthly, by using laboratory protocols recommended by the WHO, results from this study allow for comparisons with other studies both nationally and internationally. Finally, risk factors were modelled considering plausible variables for STH transmission and taking into account within-school clustering.

Since the present study was nested into a parent study, which aimed to compare STH prevalences in boys and girls and to assess gender-specific risk behaviours, a limitation of this study is that the sample size was calculated considering only the parent study's objectives. This might have limited the power of the present study. Another limitation in this study could stem from the fact that by analysing a single stool sample, STH prevalence may have been underestimated. However, considering the high prevalence obtained, we assume this underestimation to be minimal. By the same token, although this study followed procedure recommended by the WHO (WHO, 1991, 1994), there is a possibility that infection intensities may also have been underestimated. Based on recent studies confirming that Kato-Katz is fairly reliable for the three STH species investigated in the present study (Krauth et al., 2012; Tarafder et al., 2010), we propose that this underestimation might have been also minimal.

CHAPTER 6: CONCLUSIONS, RECOMMENDATIONS, FUTURE RESEARCH AND FINAL REMARKS

6.1: Conclusions

This is the first comprehensive investigation of risk factors involved in STH infection and intensity in schoolchildren living in rural communities in Honduras. The findings obtained through this research have the potential to inform STH control efforts in Honduras.

This study confirms that in Honduras, STH infections are highly prevalent and therefore an unresolved public health problem. In addition, this study demonstrated that moderate to heavy infections as well as polyparasitism are common in children living in rural Honduras. The fact that this study detected important differences in the deworming schedule regimen in the investigated communities emphasizes the challenging of implementing nationwide strategies in a resource-poor country.

Importantly, this study has identified a number of risk factor associated with STH transmission and infection intensity. Individual and familial factors such as gender, socio-economic status, STH awareness, household sanitary conditions, earthen floors and walking barefoot outdoors were significantly associated with STH infection.

Furthermore, this investigation revealed that factors beyond the individual and familial domain play a key role in both, the prevalence and infection intensity of these parasitoses in the studied children. Namely, the sanitary conditions of the schools as well as the deworming schedule implemented by them were found strongly associated with the transmission of these helminthiases

6.2: Recommendations

It is important for Honduras to continue and expand PC coverage to reach the desired 75% of children at risk for STH infection. At the same time, it is fundamental that monitoring and evaluation of the PC program is implemented to ensure that the entire territory is adequately covered. As revealed by the present investigation, the studied communities, even though enrolled in the national deworming program, had dissimilar treatment schedules affecting STH transmission and intensity.

In addition, reliance on PC alone might not lead to a sustained decrease in morbidity as the WHO advocates. In developing countries, a variety of challenges preventing the optimal performance has been identified. Therefore, interventions with an integrated approach would be beneficial (Alum et al., 2010; Kabatereine et al., 2010; Knopp et al., 2011b).

In light of the results of the present study, showing hyperendemic transmission in the studied communities in Olancho, it would be valuable to identify geographical areas of high priority for targeted interventions. Mapping out STH prevalence by parasite species at the second administrative level (municipalities) would help recognizing these areas.

The above recommendations are applicable to the centralized public health authorities and their implementation is subject to the availability of resources, technical assistance and political will. They might not be easily achieved or sustained. There are, however, other recommendations that can be suggested for an immediate implementation. Firstly, the role of local municipalities in their population's health should not be overlooked. Access to safe water and proper sanitation can and should be an integral part of their political agenda. Feasible alternatives are available to increase environmental sanitation and population hygiene. Community participation is crucial to enhance living standards and reduce health threats.

At the grassroots level, schools' sanitary conditions could be greatly improved with the participation of teachers, parents and pupils. The present study identified that increase awareness about parasites and good sanitary conditions in schools were important in reducing the odds of infection. These are modifiable factors that if implemented have the potential of reducing STH prevalence and transmission. Adequate sanitary facilities, safe water and soap for proper handwashing should be secured for these children. Likewise, the school system has a key role to play in keeping accurate registries of their pupils' deworming. A simple way to do this is providing every child with a "deworming history card" to prevent either under or overtreatment.

6.3: Future research

Given the importance of STH infections and the ongoing control programs for these parasitoses in Honduras, it is of utmost importance that implementation research is done to ensure their adequate performance. At the same time, in light of the scarcity of STH research, it is necessary that researchers dedicate efforts to provide scientific evidence to inform policy and practice as it pertains to STH control and their impact in human health. Knowledge gaps exist in regards to the impact of STH infection and polyparasitism in the nutritional, immunological and cognitive status of children, infection risk factors, differential distribution by ecological areas and potential zoonotic transmission. Drug efficacy and potential emergence of resistance to benzimidazoles should be also addressed in future research.

In addition to observational and prevalence studies, longitudinal investigations could be useful to fill the knowledge gaps described above. For example, a prevalence study to obtain baseline data (*e.g.,* STH prevalence, infection intensity, nutritional status, immunological profile), could be followed by a randomized control trial to study treatment efficacy and re-infection rate as well as other interventions (*e.g.,* health education, behaviour change). At the same time, a 2 – 3 year longitudinal study may help elucidate infection/re-infection risk factors at play and the health benefits of regular deworming. Since children living in impoverished areas in endemic countries are the most vulnerable for STH infections, a priority could be given to undertake these studies in schoolchildren living in rural communities. However, it would be ideal also to conduct these studies in pre-school children and their families.

The present study identified several risk factors for STH infection among the participants, some of which were related to personal beliefs and health behaviours. It would be worth gaining a deeper understanding of the real life context, perspectives and cultural idiosyncrasy influencing such beliefs and behaviours. Integrating qualitative studies with quantitative research could enhance our current understanding of forces at play in STH transmission and infection intensity.

6.4: Final remarks

The study presented in this thesis reveals an extremely high prevalence of STH infections in the study communities and provides a glimpse of the infection risk factors operating in the study population. We identified that school deworming schedule and school hygienic conditions were strongly associated with both STH transmission and infection intensity. Previously, we demonstrated that in the same population, these intestinal parasites had a negative impact in the children's nutritional status, especially when multiple infections occurred within the same child (Sanchez et al., 2013).

While these findings show that STH infections are a public health problem in Honduras, they present an encouraging future as long as control efforts continue uninterrupted. Improving hygienic conditions at school level may contribute to a sustainable reduction in STH prevalence and their associated morbidity (WHO, 2012).

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APPENDIX A –Ethics clearance. Brock University

Brock University
Research Ethics Board
Tel: 905-688-5550 ext. 3035
Email: reb@brocku.ca

Certificate of Ethics Clearance for Human Participant Research

DATE:	1/13/2011
PRINCIPAL INVESTIGATOR:	Theresa Gyorkos, Ana Sanchez - Child & Youth Studies
FILE:	10-161 – Sanchez/Gyorkos
TYPE:	Faculty Research/ Masters Thesis/Project
STUDENT:	Jose Antonio Gabrie & Mary-Theresa Usuanlele
SUPERVISOR:	Ana Sanchez & Theresa Gyorkos

TITLE: Soil - Transmitted Helminth Infections in Honduran School Children

ETHICS CLEARANCE GRANTED

Type of Clearance: NEW   Expiry Date: 1/31/2012

The Brock University Research Ethics Board has reviewed the above named research proposal and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement. Clearance granted from **1/13/2011 to 1/31/2012**.

The Tri-Council Policy Statement requires that ongoing research be monitored by, at a minimum, an annual report. Should your project extend beyond the expiry date, you are required to submit a Renewal form before **1/31/2012**. Continued clearance is contingent on timely submission of reports.

To comply with the Tri-Council Policy Statement, you must also submit a final report upon completion of your project. All report forms can be found on the Research Ethics web page.

In addition, throughout your research, you must report promptly to the REB:

a) Changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) All adverse and/or unanticipated experiences or events that may have real or potential unfavourable implications for participants;
c) New information that may adversely affect the safety of the participants or the conduct of the study;
d) Any changes in your source of funding or new funding to a previously unfunded project.

We wish you success with your research.

Approved:

Michelle McGinn, Chair
Research Ethics Board (REB)

Note: Brock University is accountable for the research carried out in its own jurisdiction or under its auspices and may refuse certain research even though the REB has found it ethically acceptable.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and clearance of those facilities or institutions are obtained and filed with the REB prior to the initiation of research at that site.

APPENDIX B –Ethics clearance. McGill University

L'Institut de recherche
du Centre universitaire de santé McGill
The Research Institute
of the McGill University Health Centre

November 23, 2010

Dr. Theresa Gyorkos
MUHC - RVH
Room V2.18
Montreal, Quebec H3A 1A1

Re: MUHC Authorization to Conduct Human Subjects Research 10-175-PED

Dear Dr. Gyorkos:

We are writing to confirm that the study titled *"Gender and Parasitic Diseases: Integrating Gender Analysis in Epidemiologic Research on Parasitic Diseases to Optimize the Impact of Prevention and Control Measures"* was submitted for all institutional reviews required by McGill University Health Centre policy.

The Pediatric (PED) Research Ethics Board (REB) has notified us that ethical approval to conduct your study has been provided.

In addition, all Site Specific Assessments (SSA) received favorable reviews and therefore, you are authorized to conduct the study at the MUHC.

Please refer to the MUHC Study Code **10-175-PED** in all future correspondence relating to this study.

Important Note: You are required to advise the MUHC once the study has been initiated. Please complete the Study Status Report to indicate the date the study became active and forward as an email attachment to the (1) PED REB, and (2) RI MUHC Central Output Coordinator at evelyn.ortega@muhc.mcgill.ca.

On behalf of the MUHC, we wish you every success with the conduct of the research.

Sincerely,

Miguel Burnier, MD, PhD
Associate Director for Clinical Research
The Research Institute of the McGill University Health Centre

cc: REB Study File
RI MUHC Study File

APPENDIX C –Ethics clearance. MEIZ-UNAH

OF-MEIZ-Dictamen-001-2011

Tegucigalpa M.D.C.

10 de febrero de 2011

DOCTORA
ANA SÁNCHEZ, PHD
INVESTIGADORA PRINCIPAL DE PROYECTO
UNIVERSIDAD DE BROCK
ST. CATHARINES, CANADÁ

Estimada Doctora Sánchez:

Reciba cordiales saludos y éxitos en sus diarias labores.
En respuesta a su solicitud de revisión del protocolo "Gender and parasitic diseases: Integrating gender analysis in epidemiologic research on parasitic diseases to optimize the impact of prevention and control measures (Working title in Honduras: Soil-Transmitted Helminth Infections in Honduran School Children)" y en mi condición de Oficial de Ética de la Maestría en Enfermedades Infecciosas y Zoonóticas le informo que, después de analizar el documento, no .he encontrado ninguna objeción ni recomiendo ninguna enmienda a este pues sus procedimientos propuestos se enmarcan en los requerimientos éticos establecidos en las directrices internacionales que regulan la investigación con seres humanos.
Sin otro particular se suscribe,

Atentamente,

DRA. VILMA ESPINOZA
OFICIAL DE ÉTICA
MAESTRÍA EN ENFERMEDADES INFECCIOSAS Y ZOONÓTICAS
ESCUELA DE MICROBIOLOGÍA, UNAH
TEGUCIGALPA, HONDURAS

c.c.Theresa Gyorkos
c.c.Maritza Canales
c.c. archivo

APPENDIX D –Invitation letter to schools

Research Project: Soil-Transmitted Helminthes Infections in Honduran School Children

Date:

To:

Re: Invitation to enroll your school in a research study

Dear School Principal,

We would like to invite your school to participate in a study about intestinal parasitic worms. This study is a collaborative effort between various institutions in Honduras and Canada.

In **Honduras** the main investigators are Maritza Canales (Professor) and Maria Mercedes Rueda (Grad student) of the School of Microbiology of the National Autonomous University of Honduras.

In **Canada** the main investigators are Dr. Ana L. Sanchez (Professor of Brock University) and Dr. Theresa W. Gyorkos (Professor of McGill University) and Mary-Theresa Usuanlele and Jose Antonio Gabrie (Grad students, Brock University).

Purpose of the study

The main purpose of this study is to explore gender determinants of worm infections in Grade 4 and 5 children attending primary schools in your areas. Additionally, we will try to determine what other factors are influencing the transmission of parasites among the participating children as well as assess the health impact of these infections.

Study procedures

If you agree that your school participates in this study, 4th and 5th grades will be visited to explain the children about the study and extend an invitation for the parents or guardians to attend an informational meeting with the purpose of explaining the importance of intestinal parasites as well as the benefits and risks of participating in this study.

The study will be conducted in two different stages as follows:

- ***Stage 1.*** This stage will take place at your school. Utilizing a questionnaire, we will ask the child some questions about their knowledge about parasites, health and health-related behaviours, etc. We will also ask the child to provide us with a stool specimen and a blood sample to check for intestinal parasites, anemia and other studies related to parasites. We will also take the child's weight and height measurements.
- ***Stage 2.*** A small subsample of children will be randomly selected for this stage in which an in-depth interview will take place. In this case, we will meet every child at his/her home and discuss topics related to his/her health and worm infections.

Ethical issues

We would like to assure you that this study has been reviewed and has received ethics approval from the Ethics Review Board at Brock University in St. Catharines, and the McGill University Health Centre in Montreal, both in Canada.

Risks and discomfort

There could be some embarrassment attached to providing stool samples and a natural fear of the pain associated with venipuncture for blood collection, but we will make all efforts to help parents and children to overcome any anxiety by stressing out the benefit of the study and the importance of knowing the children's health status.

Potential benefits

Because the school is located in an area where parasites are common, children are at risk of developing worm infections. The worm parasites can cause delays and impairment in the physical and intellectual growth and development of children. The direct benefit to every child from participating in this study is that he/she will be treated for these intestinal parasites if they are found to be infected, and will be treated for the anemia if that condition is found in the child at that time. This will improve their health. Additionally, they can implement prevention measures (improve hygiene, modify behaviour, etc.) and avoid future infections and potential illness.

Furthermore, the school system could use the information from this study to support continued deworming programs in Honduras.

Additionally, a workshop will be held once data collected is completed to teach the children how to wash their hands properly and other hygiene concepts.

Confidentiality

All information obtained during this study will be kept strictly confidential. Parents and children's names will not be given to anyone outside of the research team and the information will be locked in a filing cabinet in the investigator's office. Only the research team will have access to the information and only after first receiving the approval of the principal investigator. The results of this study will be published but no names will be used at any time. Participants' identity will not be revealed in the combined results. In order to verify the research data, the Quality Assurance Officers from Brock University and MUHC Research Ethics Boards may review these records.

Voluntary participation and withdrawal from this study

The participation of every child in this study is strictly voluntary. A child may refuse to participate and also may discontinue their participation at any time without explanation, and without penalty or loss of benefits to which they are otherwise entitled. Doing that, the child will suffer no prejudice regarding the medical care or participation in any other research study. Parents will be informed of any new findings that may affect their child willingness to continue his/her participation.

Cost and compensation

There are no costs associated to either school or participants in this study. If the child is infected they will be offered a single dose of Albendazole 400 mg, free of charge, (as recommended by the World Health

Organization). If anemia is detected, the proper treatment will be dispensed trough the health authorities in your community, free of charge too.

Neither children nor their parents will be given any money to participate in this study. However, a small token will be given as a sign of appreciation.

We are looking forward to your response. Please do not hesitate to contact the Coordinator of the study if you have any questions regarding.

Sincerely,

Maritza Canales, MSP
Coordinator of the Study in Honduras
Academic Coordinator of the Master Program in Infectious and Zoonotic Diseases
Associate Professor
School of Microbiology, Tegucigalpa
National Autonomous University of Honduras (UNAH)
Office phone: 2252-8089
Cell phone: 9487-0165
E-mail: <marygchn@yahoo.com>

APPENDIX E – School principal authorization form

Estudio de investigación: Parásitos intestinales en niños escolares de Honduras

Aceptación de Participación de la Escuela en el Estudio de Investigación

Yo ______________________________ en calidad de director(a) de la Escuela ____________________ ______________________________ en la Comunidad ______________________________ , perteneciente al municipio de ______________________________ en el departamento de __________ ______________________________ , doy fe que he sido informado(a) detalladamente acerca del estudio de investigación denominado **"Parásitos intestinales en niños escolares de Honduras"**, que será desarrollado en distintas escuelas de varias comunidades de nuestro país, por la Escuela de Microbiología de la Universidad Nacional Autónoma de Honduras (UNAH) y las Universidades de Brock y McGill de Canadá.

Habiendo conocido y evaluado los potenciales beneficios y riesgos que dicho estudio conlleva, y luego de haber tenido la oportunidad de realizar las preguntas pertinentes y de obtener respuesta satisfactoria a todas ellas, por este medio **acepto** que nuestra Escuela participe en el estudio ya mencionado y que los investigadores puedan acercarse a nuestros alumnos y sus padres para invitarlos a participar en el estudio.

Queda entendido, sin embargo, que esta aceptación a nivel de la escuela **no conlleva obligación alguna** hacia los padres de familia ni hacia los niños que asisten a nuestra escuela. La participación de los niños en este estudio será absolutamente voluntaria y sólo podrá darse una vez que se haya obtenido el consentimiento previo de sus padres o encargados y que el niño(a) acepte participar. Asimismo, el niño(a) será capaz de retirarse del estudio, si así lo desea, en cualquier momento, sin ninguna explicación ni represalia de ningún tipo.

______________________________ ____________________

Firma del director(a) Fecha (día/mes, 2011)

Version: February 10, 2011
Page **1** of **1**

APPENDIX F – Parental / 3rd party consent

Research Project: Soil-Transmitted Helminthes Infections in Honduran School Children

PARENTAL / 3RD PARTY CONSENT

Date:

Introduction:
Hello, we would like to invite your children to participate in a research study about parasites and intestinal worms in school children. In order for us to invite and enroll your children in this study, we first need to talk to you and obtain your permission. So we would like to tell you more about us and what is involved in the study.

Who are we?
We are a group of professors and students from Canada and Honduras interested in conducted this study. Our names and institutions are as follows:
In **Honduras** the main investigators are Dr. Maritza Canales (Professor) and Maria Mercedes Rueda (Grad student) of the School of Microbiology of the National Autonomous University of Honduras. Dr. Canales is the Honduran Project Coordinator.
In **Canada** the main investigators are Dr. Ana Sanchez (Professor at Brock University) and Dr. Theresa W. Gyorkos (Professor at McGill University) and Mary-Theresa Usuanlele and Jose Antonio Gabrie (Grad students, at Brock University).

Invitation:
We thank you for your presence here today. The reason why we are here is because we would like to invite you and your child who attends 4th or 5th grade at the school to participate in a study about parasites and intestinal worms.

Before you decide to participate, it is important that you understand the study including its risk and benefits to make an informed decision. We invite you to ask questions if there is anything that you do not understand. This document we are reading aloud is called a consent form. Because it is actually for you to give permission for your child, the form is called Parental/3rd Party Consent form.

We will give a copy of this form to each and everyone of you for you to keep but we also want to read it aloud today to make sure everybody understands it. If you decide to allow your child to participate in the study we want to propose, it should be because you fully understand the study and have come to an informed decision.

Purpose of the study
Like we said, we would like to know more about parasites and intestinal worms in school children. We would like to know why and how children are getting intestinal worms and if being a boy or a girl has anything to do with being infected. We would like to do the study with children in Grades 4th and 5th because they are at high risk of having worms and also because they are old enough to understand the study and answer some questions we would like to ask them.

What is involved in the study?

If you agree to participate, you will be asked to grant us permission to meet your child on two occasions within the next month. We will need approximately 30 minutes of his/her time in our first meeting and about 45-60 minutes for the second visit. The first meeting will be at the school and the second at your house, if you give us permission.

Now we would like to give you more details about the study:

The study will be conducted in two different stages as follows:

- ***Stage 1.*** This stage will take place at your child's school. First we will take your child's weight and height measurements, including a measurement of their waist and hip circumference. Then, we will ask your child some questions about his/her health and health habits and other questions about parasites, the games they play, if they help around the house, etc. This period of questions will last about 20 minutes. We will also ask your child to provide us with a stool specimen and a blood sample to check for intestinal parasites, anemia and other studies related to parasites. In total we would need about 30 minutes of your child's time. Of course we will ask the teacher for a moment when we don't interfere with your child's activities at school.

- ***Stage 2.*** Only a few children will be selected for the second stage. Your child may be selected for this stage in which an in-depth interview will take place. In this case, we will meet your child at your home and discuss topics related to his/her health, beliefs, activities, and other things that may be related to worm infections. This visit will require 45-60 minutes of your child's time.

Ethical issues

We would like to assure you that this study has been reviewed and has received ethics approval from impartial professionals in Honduras and in Canada. More specifically, the Ethics Review Board at Brock University in St. Catharines, and the McGill University Health Centre in Montreal, both in Canada have approved the study. Additionally, it has been approved by the principal at your child's school. These approvals mean that we will do nothing harmful to you or your children. Not only we want to do good things but also it is our obligation to conduct ourselves in a way that we don't cause any problems as a result of the study.

Risks and discomfort

This study does not pose serious risks to your child. Your child may feel a little embarrassed of providing a stool samples but we would like to say that we are very familiar with this kind of samples and we only are interested in finding parasites in them.

Also, you may have concerns about your child giving a blood sample because getting a needle is a little painful. But we want to reassure you that the pain is temporary and will go away in a few minutes. We are very experience in taking blood samples so we will not cause any more pain than a little prick.

We want to assure you that we will not give any of the stool or blood sample to anybody else, for any purpose. We may send a little bit of the samples to Canada so they can examine them in their laboratories.

Potential benefits

Because you live in an area with all the conditions for worm transmission, your child is at risk of having worm and other parasitic infections. Parasites can cause children not to grow as fast as they should, because they still nutrients and blood. Parasites can also cause poor school performance because children with worms are tired and don't learn as much as children without parasites.

The direct benefit to your child from participating in this study is that if they are found to be infected, we will offer you treatment for free. And if they have anemia or other parasites, we will provide treatments to the health care centre so you can get treatment there. These treatments will improve his/her health.

The aggregated results from the study will be presented to all of you in a meeting similar to this one. We will present recommendations as to how reduce this risk of worm infections in children in particular and community members in general.

Furthermore, we will use the information from this study to support continued deworming programs in your area. This means that we will speak with health and education authorities about developing school-based deworming programs.

Confidentiality

All information obtained during this study will be kept strictly confidential. Your name and the name of your child will not be given to anyone outside of the research team and the information will be locked in a filing cabinet in the investigator's office. Only the research team will have access to the information and only after first receiving the approval of the principal investigator. The results of this study will be published but no names will be used at any time. Your identity will not be revealed in the combined results. In order to verify the research data, the Quality Assurance Officers from Brock University and MUHC Research Ethics Boards may review these records. By signing this consent form, you give us permission to release information regarding to your participation in this study to these individuals.

Voluntary participation and withdrawal from this study

The participation of your child in this study is strictly voluntary. You may refuse your child's participation and you may discontinue his/her participation at any time without explanation, and without penalty or loss of benefits to which you are otherwise entitled. If you discontinue your child's participation, your child will suffer no prejudice regarding the medical care or participation in any other research study. You will be informed of any new findings that may affect your child willingness to continue your participation. If your child's participation is discontinued at any point in the study, we may want to keep the information you already gave us, if that's ok with you. In that case, we will still provide laboratory results if your child were to be infected and make treatment available for you at the health centre.

Cost and compensation

There are no costs associated with your participation in this study. Your child will receive one dose of Albendazole at school if he/she is found to be infected, free of charge. In case of your child be found with anemia, the proper treatment will be dispensed trough the health authorities in your community, free of charge too. Neither you nor your child will be given any money to participate in this study. However, a small token will be given to your child as a sign of appreciation.

Contact persons

If you have any questions regarding the study, you can contact the Project Coordinator in Honduras, Dr. Maritza Canales. She works at the School of Microbiology of the National Autonomous University of Honduras (UNAH) in Tegucigalpa. Her phone numbers are: office Tel. 2252-8089; Cell. 9487-0165. If you or anybody you know have access to email, you could e-mail her at <marygchn@yahoo.com>.

Declaration of consent

I understand the contents of this **Consent Form**, and I agree that my child participates in this research study. I understand that not all the children who participate in this study will be selected for the Stage 2 and I agree that my child participates in Stage 2 if he/she is selected to do so. I have had the opportunity to ask questions in an information session and all my questions have been answered to my satisfaction. I have been given sufficient time to consider the above information and to seek advice if I choose to do so. By signing this consent form, I am not given up any of my legal rights, just agreeing that my child participates in the research study.

I agree that my child participates in

First stage of the study	☐ Yes	☐ No
Second stage of the study	☐ Yes	☐ No

I agree that my child provides

Stool sample	☐ Yes	☐ No
Blood sample	☐ Yes	☐ No

____ School_______________ CODE: __________________

Child's name

___________________________	___________________________	___________________
Parent's/guardian's name	Parent's/guardian's signature	Date (day/month, 2011)
___________________________	___________________________	___________________
Witness's name	Witness's signature	Date (day/month, 2011)

APPENDIX G – Informed assent for children

Research Project: Soil-Transmitted Helminthes Infections in Honduran School Children

INFORMED ASSENT FOR CHILDREN

Date:

Introduction:

Hello, we are here because your parents gave us permission to talk to you and invite you to participate in a research study about parasites and intestinal worms in school children. Eventhough your parents gave us permission, we need to know if YOU agree to participate in the study. So we would like to tell you more about us and what is involved in the study.

Who are we?

We are a group of professors and students from Canada and Honduras interested in conducted this study. Our names and institutions are as follows:

In **Honduras** the main investigators are Dr. Maritza Canales (Professor) and Maria Mercedes Rueda (Grad student) of the School of Microbiology of the National Autonomous University of Honduras. Dr. Canales is the Honduran Project Coordinator.

In **Canada** the main investigators are Dr. Ana Sanchez (Professor at Brock University) and Dr. Theresa W. Gyorkos (Professor at McGill University) and Mary-Theresa Usuanlele and Jose Antonio Gabrie (Grad students, at Brock University).

Invitation:

We thank you for your presence here today. The reason why we are here is because we would like to invite you to participate in a study about parasites and intestinal worms. I am going to spend a few minutes to telling you about our project, and then I am going to ask you if you are interested in taking part in the project.

Why we are meeting with you?

We want to tell you about a study that involves children like yourself in schools of your area. We want to see if you would like to be in this study too. We want to find out ways to get rid of the intestinal parasites that can cause harm to your health.

What will happen to you if you are in the study?

If you decide to take part of this study there are some different things we will ask you about your health and habits. You will meet with one of our interviewers. We will measure your weight and height, your waist and your hip circumference. We will also ask you to give us a small stool specimen and blood sample. We might also visit you at your home at a later time to have a chat about your health and intestinal worms. While doing these things all you have to do is try your best. It will take you about 30 minutes to answer our questions and to give us your blood sample on our first visit to your school. At that day you will have to bring the stool sample at school in a special container that we will provide to you. If we meet a second time, it will be at your home, and the chat should last about an hour.

Are there good things and bad things about the study?

One of the good things is that you will get rid of your parasites and if you have anemia you will get treatment too. This means that you will probably feel better and be able to pay more attention in school. Another good thing is that we will use what we find out from this study to help school children around the world who also have parasites.

There are not really bad things in this study, but we understand that you can feel anxious about providing the stool and getting a needle to give a blood sample. It is important that you know that scientists can examine these types of samples in order to understand your health status. Stools are a natural byproduct of our bodies and their examination is a routine procedure if we want to know if the person has parasites.

When we take your blood sample you will feel some pain, but maybe you had your blood sample taken before? In this case you know that this pain will go away in a few minutes. Sometimes you can get a small bruise on the site where the needle was applied but this will also go away in a few days. It is important you understand that some parasites can give anemia and the only way to know if you have anemia is by examining your blood. So you can decide if you want to have your blood examined or not. You can decide if you only want to give us the stool sample or both. Either way, it's ok with us.

We want to assure you that we will not give any of the stool or blood sample to anybody else, for any purpose. We may send a little bit of the samples to Canada so they can examine them in their laboratories.

Will you have to answer all of the questions and do everything you are asked to do?

The questions we will ask you will be easy to answer but if you don't want to answer, then you don't have to. Just tell us. Also, if you don't want to do something else we ask you to do, it's all right; just tell us that you don't want to do it. Nothing bad will happen if you don't want to tell us something or if you don't want to do something we have asked you to do. There is no right or wrong answer to any of the questions.

Who will know that you are in the study?

The things you tell us and any information that we have about you will be kept secret in a locked place. Only the researchers will be allowed to see it. Your teachers will not see it and your parents won't see it. When we talk about this study, we will never use your name.

Voluntary participation and withdrawal from this study

Your participation in this study is strictly voluntary. You may enroll now and refuse to continue later. If that is the case, you don't have to give us too many explanations, just tell us you don't want to continue. If you drop out from the study, nothing bad will happen to you. Nobody will reprimand you or anything like that (including your parents or teachers).

If you no longer wanted to be part of the study, we may want to keep the information you already gave us, if that's ok with you. In that case, we will still give your parents your laboratory results, especially if you had parasites. If you did, we will make treatment available for you at the health centre.

Do you have any questions?

You can ask questions at any time. You can ask now or you can ask later. You can talk to me or you can talk to someone else at any time during the study.

If you have questions after we are gone, you can tell your parents or your teacher to help you contact the Project Coordinator in Honduras. Her name is Dr. Maritza Canales. She works at the School of Microbiology of the National Autonomous University of Honduras (UNAH) in Tegucigalpa. Her phone numbers are: office Tel. 2252-8089; Cell. 9487-0165. if you or anybody you know has access to email, you could e-mail her at <marygchn@yahoo.com>.

Declaration of Assent

I have been explained the details of this research study and I understand it. I have had the opportunity to ask questions and all of them were answered satisfactorily. My participation in this study is free and voluntary. I know that I can withdraw from this study at any time if I want to, without any bad consequences to me. I agree to participate.

The child provides verbal assent to:

Being interviewed at school for the questionnaire	☐ Yes ☐ No
Being interviewed at home if selected	☐ Yes ☐ No
Providing a stool sample	☐ Yes ☐ No
Providing a blood sample	☐ Yes ☐ No
Authorizing researchers to keep their data even if withdraw from the study	☐ Yes ☐ No

_____________________________________ School_______________ CODE: __________________

Child's name

___________________________	___________________________	___________________
Researcher's name	Researcher's signature	Date (day/month, 2011)
___________________________	___________________________	___________________
Witness's name	Witness's signature	Date (day/month, 2011)

APPENDIX H – Child questionnaire

QUESTIONNAIRE – Children (HONDURAS)
ENCUESTA – Niños (HONDURAS)

Project title: Soil-Transmitted Helminthes Infections in Honduran School Children
Parásitos Intestinales en niños escolares de Honduras

IDENTIFICATION OF SCHOOL, CHILD, INTERVIEWER
IDENTIFICACIÓN DE LA ESCUELA, NIÑO(A), ENTREVISTADOR

SCHOOL: **ESCUELA** ____________________	LAST, FIRST NAME OF CHILD **APELLIDO, NOMBRE DEL NIÑO(A):** _______________, _______________	INTERVIEWER **ENTREVISTADOR:** ____________________
LAST, FIRST NAME OF TEACHER **APELLIDO, NOMBRE DEL MAESTRO(A):** __________, __________	IDENTIFICATION CODE **CÓDIGO DE IDENTIFICACIÓN:** - - (SCHOOL INITIALS - CLASS – CHILD'S CODE) **(INICIALES ESCUELA - AULA - CÓDIGO NIÑO)**	DATE OF INTERVIEW (dd/mm/yyyy) **FECHA DE ENTREVISTA (dd/mm/aaaa):** / /2011
GRADE/CLASS **GRADO/AULA:** ______ / ______		

ELIGIBILITY CRITERIA
CRITERIOS DE ELEGIBILIDAD

A	Obtained parent/ guardian consent? **¿Se obtuvo consentimiento de sus padres o encargados?**	☐ Yes **Sí** *Continue to question B* ***Pase a la pregunta B***	☐ No **No** *Exclude participation* ***Excluya su participación***
B	Obtained child assent? **¿Se obtuvo el asentimiento del niño?**	☐ Yes **Sí** *Continue to question 1* ***Pase a la pregunta 1***	☐ No **No** *Exclude participation* ***Excluya su participación***

BASIC INFORMATION
INFORMACIÓN BÁSICA

1	Indicate in which shift the child attends school: **Jornada de clase:**	☐ Morning **Mañana** (1)	☐ Afternoon **Tarde** (2)	☐ Night **Noche** (3)
2	What is the child's sex? **¿Sexo?**	☐ Male **Masculino** (1)	☐ Female **Femenino** (0)	
3	When is the child's birthday? (dd/mm/yyyy) **¿Fecha de nacimiento? (dd/mm/aaaa)**	____/____/____	☐ *Don't know* ***No sabe*** (99)	Age **Edad** ________
4	Child weight **Peso del niño**	________ kg **kg**	Scale # **Balanza #**	
5	Child height **Estatura del niño**	________ cm **cm**		
6	Does the child have dirty finger nails? **¿El niño(a) tiene las uñas sucias?**	☐ Yes **Sí** (1)	☐ No **No** (0)	
7	Is the child wearing shoes/sandals? **¿El niño lleva puestos zapatos o sandalias?**	☐ Yes **Sí** (1)	☐ No **No** (0)	

PERCEPTION/KNOWLEDGE OF PARASITES
PERCEPCIÓN/NIVEL DE CONOCIMIENTO SOBRE LOS PARÁSITOS

8	Do you know what (parasitic) worms are? **¿Sabes qué son las lombrices intestinales?**	☐ Yes **Sí** (1) ☐ No **No** (0)
9	**A-** Do you think that you could get worms? **¿Crees que podrías infectarte con lombrices intestinales?**	☐ Yes **Sí** (1) ☐ No **No** (0) ☐ *Don't know* ***No sabe*** (99)
	B- If yes, explain. **En caso de sí, explicar** ☐ *Not applicable* ***No aplica (NA)***	______ __________________________
10	**A-** Do you think that worms are good for you? **¿Crees que es bueno tener lombrices intestinales?**	☐ Yes **Sí** (1) ☐ No **No** (0) ☐ *Don't know* ***No sabe*** (99)
	B- If yes <u>or</u> no, why? **En caso de sí <u>o</u> no, ¿por qué?** ☐ *Not applicable* ***No aplica (NA)***	______ __________________________
11	**A-** Do you try to prevent getting worms? **¿Tratas de evitar infectarte con lombrices?**	☐ Yes **Sí** (1) ☐ No **No** (0) ☐ *Don't know* ***No sabe*** (99)
	B- If yes, how? **En caso de sí ¿cómo?** ☐ *Not applicable* ***No aplica (NA)***	1) ________________ 2) ________________ 3) ________________ 4) ________________
	C- Do you know of any other way(s) to prevent getting worms? **¿Conoces alguna otra forma de evitar infectarte con estas lombrices?**	☐ Yes **Sí** (1) ☐ No **No** (0)
	D- If yes, how? **En caso de sí ¿cómo?** ☐ *Not applicable* ***No aplica (NA)***	1) ________________ 2) ________________ 3) ________________ 4) ________________
12	**A-** Do you know how you get worms? **¿Sabes cómo se transmiten las lombrices intestinales?**	☐ Yes **Sí** (1) ☐ No **No** (0)
	B- If yes, how? **En caso de sí ¿cómo?** ☐ *Not applicable* ***No aplica (NA)***	1) ________________ 2) ________________ 3) ________________ 4) ________________
13	Have you ever had worms? **¿Has tenido alguna vez lombrices intestinales?**	☐ Yes **Sí** (1) ☐ No **No** (0) ☐ *Don't know* ***No sabe*** (99)

RISK FACTOR CATEGORY 1: HOUSEHOLD
FACTOR DE RIESGO CATEGORÍA 1: CASA

14	**A**- What community do you live in? **¿En qué comunidad /localidad vives?**	Community **Comunidad:** ....
	B- On what street/sector/lot is your house? **¿En qué calle o sector o lote se ubica tu casa?**	Address **Dirección:** .... ☐ *Don't know* ***No sabe*** (99) ☐ *Not applicable* ***No aplica (NA)***
	C- How do you usually get to school? **¿Cómo vienes a la escuela la mayor parte del tiempo?**	☐ Walk **Camina** (0) ☐ Car **Carro** (2) ☐ Bus **Autobús** (1) ☐ Other **Otro :** ____________________ (3)
	D- How long does it take you to get to school (in minutes)? **¿Cuánto tiempo tardas en llegar desde tu casa a la escuela (en minutos)?**	_ _ _ _ _ _ minutes **minutos** ☐ *Don't know* (99) ***No sabe***
15	What is the floor of your home made of? **¿De qué material está hecho el piso de tu casa?**	☐ Tiles **Ladrillo** (0) ☐ Earth **Tierra** (2) ☐ *Don't know No sabe* (99) ☐ Cement **Cemento** (1) ☐ Other **Otro :** ____________________ (3)
16	In your house, do you cook with gas, kerosene, carbon or firewood? **¿Con qué tipo de estufa cocinan en tu casa?**	☐ Gas **Gas** (0) ☐ Carbon **Carbón** (2) ☐ *Don't know* ***No sabe*** (99) ☐ Kerosene **Kerosén** (1) ☐ Firewood **Leña** (3) ☐ Other **Otra**_____ (4)
17	Does your house have **¿Tienes en tu casa?:**	**A** - Electrical energy? **¿Energía eléctrica?** ☐ Yes **Sí** (1) ☐ No **No** (0) ☐ *Don't know* ***No sabe*** (99) **B** - A radio? **¿Radio?** ☐ Yes **Sí** (1) ☐ No **No** (0) ☐ *Don't know* ***No sabe*** (99) **C**- A television? **¿Televisor?** ☐ Yes **Sí** (1) ☐ No **No** (0) ☐ *Don't know* ***No sabe*** (99) **D**- A refrigerator? **¿Refrigeradora?** ☐ Yes **Sí** (1) ☐ No **No** (0) ☐ *Don't know* ***No sabe*** (99)

18	**A**- Do you have potable water in your house (tap water)? **¿Tienes agua potable en tu casa (agua de la llave)?**	☐ Yes **Sí** (1) ☐ No **No** (0) ☐ *Don't know* ***No sabe*** (99)
	B- If no, where do you get water for your house? **¿En caso que no, de dónde se abastecen de agua en tu casa?** ☐ *Not applicable* ***No aplica (NA)***	☐ Neighbor's tap **Llave donde un vecino** (0) ☐ River **Río** (1) ☐ Well **Pozo** (2) ☐ Water truck **Carro cisterna** (3) ☐ Tank **Tanque** (4) ☐ Public pool **Pila o llave pública** (5) ☐ Other **Otro** ____________ (6) ☐ *Don't know* ***No sabe*** (99)
19	In your house, do you drink water directly or treated (boiled/ chemicals/filtered)? **¿En casa, toman agua tratada (hervida, clorada o filtrada) o no?**	☐ Not treated **No tratada** (1) ☐ Treated **Tratada** (0) ☐ *Don't know* ***No sabe*** (99)
20	**A**- Do you have a flushable toilet? **¿Tienes servicio sanitario (inodoro)?**	☐ Yes **Sí** (1) ☐ No **No** (0) ☐ *Don't know* ***No sabe*** (99)
	B- Do you have a latrine? **¿Tienes letrina?**	☐ Yes **Sí** (1) ☐ No **No** (0) ☐ *Don't know* ***No sabe*** (99)
21	How many people usually live in your house? **¿Cuántas personas viven en tu casa habitualmente?**	**A**- Total #: **# Total:** ___________ **B-** Mother? **¿Madre?** ☐ Yes **Sí** (1) ☐ No **No** (0) **C**- Father? **¿Padre?** ☐ Yes **Sí** (1) ☐ No **No** (0) **D-** Grandmother? **¿Abuela?** ☐ Yes **Sí** (1) #:_____ ☐ No **No** (0) **E-** Grandfather? **¿Abuelo?** ☐ Yes **Sí** (1) #:_____ ☐ No **No** (0) **F-** Girls < 5 years old? **¿Niñas < 5 años?** ☐ Yes **Sí** (1) #:_____ ☐ No **No** (0) **G-** Boys < 5 years old? **¿Niños < 5 años?** ☐ Yes **Sí** (1) #:_____ ☐ No **No** (0)

22	Do you do any of the following chores on a regular basis? **¿Haces regularmente algunas de los siguientes oficios en la casa?** ☐ *No chores* ***Ningún oficio** (0)*	☐	Cooking **Cocinar**	(1)	☐	Cleaning the toilet/latrine **Limpieza del indoor/letrina**	(5)
		☐	Changing diapers **Cambiar pañales**	(2)	☐	Cleaning (other than toilet/latrine) **Limpieza de la casa**	(6)
		☐	Taking care of younger children **Cuidar niños menores**	(3)	☐	Laundry (cleaning clothes) **Lavar ropa**	(7)
		☐	Taking care of people who are sick **Cuidar enfermos**	(4)	☐	Working in the field **Trabajo en el campo**	(8)
		☐	Other: **Otro:** ______________________				(9)

			A- Animal? **¿Animal?**		B- Take care (eg. feed/water)? **¿Lo cuida?**		C- Play with? **¿Juega con él?**	
23	Do you have any domestic animal(s) in your home? (If yes, do you take care and/or play with the animal(s)?) **¿Tienen animales domésticos en tu casa? (En caso de sí, ¿lo cuida y/o juega con él?)** ☐ No animal **Ningún animal**	i- Dog **Perro**	☐ Yes **Sí** (1)	☐ No **No** (0)	☐ Yes **Sí** (1)	☐ No **No** (0)	☐ Yes **Sí** (1)	☐ No **No** (0)
		ii- Cat **Gato**	☐ Yes **Sí** (1)	☐ No **No** (0)	☐ Yes **Sí** (1)	☐ No **No** (0)	☐ Yes **Sí** (1)	☐ No **No** (0)
		iii- Pig **Cerdo**	☐ Yes **Sí** (1)	☐ No **No** (0)	☐ Yes **Sí** (1)	☐ No **No** (0)	☐ Yes **Sí** (1)	☐ No **No** (0)
		iv- Chicken **Pollos**	☐ Yes **Sí** (1)	☐ No **No** (0)	☐ Yes **Sí** (1)	☐ No **No** (0)	☐ Yes **Sí** (1)	☐ No **No** (0)
		v- Duck **Pato, ganzo**	☐ Yes **Sí** (1)	☐ No **No** (0)	☐ Yes **Sí** (1)	☐ No **No** (0)	☐ Yes **Sí** (1)	☐ No **No** (0)
		vi- Cattle **Vacas, bueyes**	☐ Yes **Sí** (1)	☐ No **No** (0)	☐ Yes **Sí** (1)	☐ No **No** (0)	☐ Yes **Sí** (1)	☐ No **No** (0)
		vii- Goat **Cabra, oveja**	☐ Yes **Sí** (1)	☐ No **No** (0)	☐ Yes **Sí** (1)	☐ No **No** (0)	☐ Yes **Sí** (1)	☐ No **No** (0)
		viii- Equine **Caballo, burro, mula**	☐ Yes **Sí** (1)	☐ No **No** (0)	☐ Yes **Sí** (1)	☐ No **No** (0)	☐ Yes **Sí** (1)	☐ No **No** (0)
		ix- Other **Otro** ________	☐ Yes **Sí** (1)	☐ No **No** (0)	☐ Yes **Sí** (1)	☐ No **No** (0)	☐ Yes **Sí** (1)	☐ No **No** (0)

RISK FACTOR CATEGORY 2: HYGIENE
FACTOR DE RIESGO CATEGORÍA 2: HIGIENE

24	Do you defecate in the open? **¿Haces pupú en la tierra o patio?**	☐	Always **Siempre**	(2)	☐	Never **Nunca**	(0)
		☐	Sometimes **A veces**	(1)	☐	*Don't know* ***No sabe***	(99)
25	Do you wipe with toilet paper after defecating - always, sometimes, or never? **¿Te limpias con papel higiénico cuando haces pupú?**	☐	Always **Siempre**	(2)	☐	Never **Nunca**	(0)
		☐	Sometimes **A veces**	(1)	☐	*Don't know* ***No sabe***	(99)

26	**A**- How many times per day, approximately, do you wash your hands? **¿Cuántas veces por día, aproximadamente, te lavas las manos?**	................times/day **veces/día**	☐ *Don't know* ***No sabe*** (99)
	B- Do you use soap when washing your hands – always, sometimes or never? **¿Usas jabón cuando te lavas las manos?** ☐ *Not applicable* ***No aplica*** *(NA)*	☐ Always **Siempre** (2) ☐ Sometimes **A veces** (1)	☐ Never **Nunca** (0) ☐ *Don't know* ***No sabe*** (99)
27	**A**- Do you wash your hands after going to the bathroom – always, sometimes, or never? **¿Te lavas las manos después de hacer pupú?**	☐ Always **Siempre** (2) ☐ Sometimes **A veces** (1)	☐ Never **Nunca** (0) ☐ *Don't know* ***No sabe*** (99)
	B- Do you use soap when washing your hands after going to the bathroom- always, sometimes or never? **¿Usas jabón cuando te lavas las manos después de hacer pupú?** ☐ *Not applicable* ***No aplica*** *(NA)*	☐ Always **Siempre** (2) ☐ Sometimes **A veces** (1)	☐ Never **Nunca** (0) ☐ *Don't know* ***No sabe*** (99)
28	**A**- Do you wash your hands before eating – always, sometimes or never? **¿Te lavas las manos antes de comer?**	☐ Always **Siempre** (2) ☐ Sometimes **A veces** (1)	☐ Never **Nunca** (0) ☐ *Don't know* ***No sabe*** (99)
	B- Do you use soap when you wash your hands before eating – always, sometimes or never? **¿Usas jabón cuando te lavas las manos antes de comer?** ☐ *Not applicable* ***No aplica*** *(NA)*	☐ Always **Siempre** (2) ☐ Sometimes **A veces** (1)	☐ Never **Nunca** (0) ☐ *Don't know* ***No sabe*** (99)
29	**A**- How many times per week do you bathe? **¿Cuántas veces a la semana te bañas?**	................times/week **veces/semana**	☐ *Don't know* ***No sabe*** (99)
	B- Do you use soap when you bathe yourself – always, sometimes or never? **¿Usas jabón cuando te bañas?** ☐ *Not applicable* ***No aplica*** *(NA)*	☐ Always **Siempre** (2) ☐ Sometimes **A veces** (1)	☐ Never **Nunca** (0) ☐ *Don't know* ***No sabe*** (99)
30	**A**- Do you bite your nails – always, sometimes or never? **¿Te comes las uñas?**	☐ Always **Siempre** (2) ☐ Sometimes **A veces** (1)	☐ Never **Nunca** (0) ☐ *Don't know* ***No sabe*** (99)
	B- Do you suck on any of your fingers (eg. suck your thumb) – always, sometimes or never? **¿Te chupas los dedos (ej. Pulgar)?**	☐ Always **Siempre** (2) ☐ Sometimes **A veces** (1)	☐ Never **Nunca** (0) ☐ *Don't know* ***No sabe*** (99)

31	For the main meal of the day, do you use your fingers to eat? **¿Comes con las manos?**	☐ Always **Siempre** (2) ☐ Never **Nunca** (0) ☐ Sometimes **A veces** (1) ☐ *Don't know* ***No sabe*** (99)

RISK FACTOR 3: ACTIVITIES
FACTOR DE RIESGO CATEGORÍA 3: ACTIVIDADES

32	**A**- Do you presently work (other than at school or home)? **¿Trabajas actualmente en otro lugar que no sea la escuela o la casa?**	☐ Yes **Sí** (1) ☐ No **No** (0) ☐ *Don't know* ***No sabe*** (99)
	B- If yes, what do you do? **¿En caso que sí, que haces?** ☐ *Not applicable* ***No aplica*** *(NA)*	Work: **Trabajo:** _
	C- If yes, how many hours/week, approximately, do you work? **¿En caso que sí, cuántas horas por semana, aproximadamente, trabajas?** ☐ *Not applicable* ***No aplica*** *(NA)*	.... hours/week **horas/semana** ☐ *Don't know* ***No sabe*** (99)
33	**A**- How often do you play outside per day, on average? **¿Cuántas horas al día, en promedio, juegas afuera?**	☐ 0 hours/day **0 horas/día** (3) ☐ 1-3 hours/day **1-3 horas/día** (1) ☐ < 1 hour/day **< 1 hora/día** (2) ☐ > 3 hours/day **> 3 horas/día** (0) ☐ *Don't know* ***No sabe*** *(99)*
	B- What games do you play outside? **¿Qué juegos haces fuera de la casa?** ☐ *None* ***Ninguno*** *(NA)*	1) _ 2) _ 3) _ 4) _
	C- Do you play in dirt- always, sometimes or never? **¿Juegas en la tierra?**	☐ Always **Siempre** (2) ☐ Never **Nunca** (0) ☐ Sometimes **A veces** (1) ☐ *Don't know* ***No sabe*** (99)
	D- Do you ever put dirt in your mouth- always, sometimes or never? **¿Te gusta comer tierra?**	☐ Always **Siempre** (2) ☐ Never **Nunca** (0) ☐ Sometimes **A veces** (1) ☐ *Don't know* ***No sabe*** (99)

33	**E-** Do you play in water – always, sometimes or never? **¿Juegas en el agua (río, quebrada, poza)?**	☐ Always **Siempre** (2) ☐ Sometimes **A veces** (1)	☐ Never **Nunca** (0) ☐ *Don't know* ***No sabe*** (99)

RISK FACTOR CATEGORY 4: WEARING SHOES
FACTOR DE RIESGO CATEGORÍA 4: LLEVAR ZAPATOS

34	Do you go outside without shoes on– always, sometimes, or never? **¿Caminas descalzo(a)?**	☐ Always **Siempre** (2) ☐ Sometimes **A veces** (1)	☐ Never **Nunca** (0) ☐ *Don't know* ***No sabe*** (99)
35	When you are at home, do you wear shoes or sandals? **¿Cuándo estás en casa, llevas zapatos o sandalias?**	☐ Shoes **Zapatos** (2) ☐ Sandals **Sandalias** (1)	☐ Neither **Ninguno** (0) ☐ *Don't know* ***No sabe*** (99)
36	In what activities do you **not** wear shoes? **¿En qué actividades del día andas descalzo(a)?** ☐ *Always wears shoes (0)* ***Siempre anda calzado(a)***	A- Activity **Actividad**: …………………………………………… B- Activity **Actividad**: …………………………………………… C- Activity **Actividad**: ……………………………………………	

RISK FACTOR CATEGORY 5: USE OF HEALTH SERVICES
FACTOR DE RIESGO CATEGORÍA 5: USO DE SERVICIOS DE SALUD

37	Can you go to the health centre and return to your home in the same day? **¿Puedes ir a un centro de salud y regresar a tu casa en el mismo día?**	☐ Yes **Sí** (1) ☐ No **No** (0)	☐ *Don't know* ***No sabe*** (99)
38	How many times have you been sick in the last year? **¿Cuántas veces estuviste enfermo(a) en el último año?**	……….times in last year **veces en el último año**	☐ *Don't know* ***No sabe*** (99)
39	How many times have you been to "the doctor" in the last year? **¿Cuántas veces te llevaron a consulta porque estabas enfermo(a) el año pasado?**	……….times in last year **veces en el último año**	☐ *Don't know* ***No sabe*** (99)
40	How many times have you been to "the doctor" in the last year for a routine check-up or vaccinations? **¿Cuántas veces te llevaron a consulta para una visita de rutina o para vacunas?**	……….times in last year **veces en el último año**	☐ *Don't know* ***No sabe*** (99)
41	Who takes care of you when you are sick? **¿Quién te cuida cuando estás enfermo(a)?**	☐ Mother **Madre** (4) ☐ Father **Padre** (3) ☐ Nobody **Nadie** (0)	☐ Other family member: **Otro familiar**: ________________ (2) ☐ Other: **Otro**: ______________________ (1) ☐ *Don't know* ***No sabe*** (99)

RISK FACTOR CATEGORY 6: HISTORY OF DEWORMING
FACTOR DE RIESGO CATEGORÍA 6: HISTORIA DE DESPARASITACIÓN

<table>
<tr><td rowspan="4">42</td><td>A- Have you ever been given treatment (traditional or modern medicine) for parasitic worms? ¿Te han dado tratamiento para las lombrices alguna vez?</td><td>☐ Yes Sí (1) ☐ No No (0) ☐ Don't know No sabe (99)</td></tr>
<tr><td>B - If yes, who? ¿En caso que sí, quién?
☐ Not applicable No aplica (NA)</td><td>☐ Teachers Maestros (1) ☐ Church Iglesia (3) ☐ Other Otro: (5)
☐ Parents Padres (2) ☐ Brigade Brigada (4) ☐ Don't know No sabe (99)</td></tr>
<tr><td>C - If yes, what did they give you? ¿En caso que sí, te acuerdas qué te dieron?
☐ Not applicable No aplica (NA)</td><td>☐ Pills Pastillas (1) ☐ Syrup Jarabe (3) ☐ Don't know No sabe (99)
☐ Herbs Hierbas (2) ☐ Other Otra ______________________ (4)</td></tr>
<tr><td>D- If yes, when was the last time you were dewormed? ¿En caso que sí, cuándo fue la última vez que te dieron ese tratamiento?
☐ Not applicable No aplica (NA)</td><td>--------- Months ago Meses ☐ Over a year ago Más de un año (13) ☐ Don't know No sabe (99)</td></tr>
</table>

Comments / Comentarios:____
____
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____
____
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Checked for completeness ☐ : by: date:
Verificó que estaba completo: **por:** ________________________ ; **fecha**: ______________
(name) **(nombre)** (dd-mm-yyyy) **(dd-mm-aaaa)**

THANK YOU FOR PARTICIPATING! ¡GRACIAS POR TU PARTICIPACIÓN!

ADDITIONAL QUESTIONS
PREGUNTAS ADICIONALES

43	**A-** Have you ever had worms? **¿Has tenido alguna vez lombrices intestinales?**	☐	Yes **Sí**	(1) ☐	No **No**	(0) ☐	*Don't know* ***No sabe***	(99)
	B- If yes, how do you know? Did you pass them in your stools? Or did you have a lab exam done? **¿Cómo sabes? ¿Te salieron con el pupú o te hicieron examen?**	☐	Passed **Expulsó**	(1) ☐	Stool exam **Examen de heces**	(2) ☐	*Don't know* ***No sabe***	(99)
	C- If passed in stools: did you pass any of these? (show round worms and tapeworms) **Si le salieron: ¿Cuál de estos te salió? *(mostrar lombrices y tenias)***	☐	Round worms **lombrices**	(1) ☐	Tapeworm **solitaria**	(2) ☐	*Don't know* ***No sabe***	(99)
	D- If yes, when was the last time? **En caso de sí, ¿Cuándo fue la última vez?**	☐	< 6 months **< 6 meses**	(1) ☐	≥ 6 months **≥ 6 meses**	(2) ☐	*Don't know* ***No sabe***	(99)
44	**A-** Do you have pigs? **¿Tienen cerdos en tu casa?**	☐	Yes **Sí**	(1) ☐	No **No**	(0) ☐	*Don't know* ***No sabe***	(99)
	B- If yes, How many? **En caso de sí, ¿Cuántos?**	-------------------------------						
	C- Do you eat pork meet? **¿Te gusta comer carne de cerdo?**	☐	Yes **Sí**	(1) ☐	No **No**	(0) ☐	*Don't know* ***No sabe***	(99)
45	How would you describe your health status? **¿Cómo describirías tu estado de salud?**	☐	Excellent (feel very well / plenty of energy) **Excelente (nunca me enfermo, me siento muy bien / con mucha energía)**	(1) ☐	Regular (tired and sleepy sometimes) **Mas o menos (paso cansado, con sueño, no me dan muchas ganar de jugar)**	(3)		
		☐	Very Good **Muy bien: a veces me enfermo, pero normalmente ando bien, con ganas jugar**	(2) ☐	Poor (sick or tired and sleepy often/affecting my studies) **Muy mal (paso enfermo todo el tiempo, y con sueño, me afecta en los estudios)**	(4)		
46	**A-** Did you miss more than 3 days from school last year? **¿Faltaste a clases por más de tres días el año pasado?**	☐	Yes **Sí**	(1) ☐	No **No**	(0) ☐	*Don't know* ***No sabe***	(99)
	B- If yes, why? **En caso de sí, ¿por qué?**	☐	Sickness **Enfermedad**	(1) ☐	Other **Otro**	(0) -------------------------------------		

APPENDIX I – Interview guideline (child questionnaire)

INTERVIEW GUIDE - CHILD QUESTIONNAIRE

FOR SELECTED QUESTIONS

Project title: Gender and parasitic diseases: Integrating gender analysis in epidemiologic research on parasitic diseases to optimize the impact of prevention and control measures

This questionnaire is to be administered to the schoolchildren

A-B	Ensure that consent/assent forms are complete (all appropriate boxes checked, signed, dated, witnessed)
1-2	Interviewer observation
3	Ask child their birthday and age
4-5	Measurement (NOTE: if more than 1 scale is being used in the study, each scale should be identified with a number, and the scale # used should be indicated in the questionnaire) Remember that each scale needs to be calibrated daily!
6-7	Interviewer observation
9B	Mark "NA" if answer to 9A was "No"
8	This question will need to be translated carefully. It is important that the culturally-appropriate words to describe parasitic worms are used. If necessary, a brief description may need to be given so that the concept of worms is adequately explained.
10B	Note that this should be answered if answer to 10A is "yes" OR "no". The NA answer is to be used only if "don't know" was the answer to 10A.
11	Note distinction between things that the child **actually does** her/himself to prevent worms (11A-B) and things that the child **knows he or she can do** (but doesn't actually do) to prevent worms (11C-D)
11B	Mark "NA" if answer to 11A was "No"
11D	Mark "NA" if answer to 11C was "No"
12	Hypothetically
12B	Mark "NA" if answer to 12A was "No"
15	This question could be changed to a different socio-economic status indicator of home, if a better one exists (eg. # of rooms), depending on each site. Check one box only (if mixed, include in "other")
16	Check one
18B	Check all that apply; Mark "NA" if answer to 18A was "No"
19	Check one; Treated refers to all options of boiling, adding chemicals or filtering water before consuming it.
21	Ask for total number of people who **usually** live in the house **AND** individually ask if their mother/father and any grandmother, grandfather and girls/boys under 5 years old live in the house (for grandmother, grandfather, girls < 5 years old and boys < 5 years old, indicate how many usually live in the house). Note girls and boys < 5 years old will likely mostly include siblings, but this could also include any other children (eg. cousins, nieces/nephews, etc.)
22	Check all that apply
23	Ask about each animal (i-ix) individually (A). For each section (i-ix), if answer to A is "Yes", ask **both** questions B and C. Do not ask questions B and C if answer to A is "No". If answer to all sections (i-ix) of A are "No", mark "No animal".
26B	Mark "NA" if answer to 26A is "0 times/day"

Version: February 7, 2011

27A-B	Cultural translation of "going to bathroom"? – this is important; Mark "NA" for B if answer to A is "Never".
28B	Mark "NA" if answer to 28A is "Never"
29B	Mark "NA" if answer to 29A is "0 times/week"
31	Refers to biggest meal of the day (may be culturally dependent – this could be changed to specify what this meal is, if obvious, in each site)
32	Paid or unpaid work
33B	List top 4 games (if more than 4 exist), or, as many games as child can think of
36	List top 3 activities if more than 3 exist
38-40	These answers can be **approximate** answers (it will be difficult to get exact answers, but approximations are fine). We want to be able to compare the number of times the child was sick to the number of times he/she sought treatment.
39-40	Refers to any health services (traditional or modern)
42	This refers to any type of treatment (eg. anthelmintics, traditional herbs, etc.)
Completeness	Have someone other than the interviewer review the entire questionnaire to ensure clarity and completion. NO QUESTIONS SHOULD BE LEFT BLANK. This person should sign and date the form.

APPENDIX J – School questionnaire

QUESTIONNAIRE – Schools

ENCUESTA – Escuelas (HONDURAS)

Project title: Soil-Transmitted Helminthes Infections in Honduran School Children

Parásitos Intestinales en niños escolares de Honduras

IDENTIFICATION

IDENTIFICACIÓN

1	Name of school **Nombre de la escuela**	....
2	Address of school **Dirección de la escuela**	....
3	Date of interview (dd/mm/yyyy) **Fecha (dd/mm/aaaa)**	/ /2011
4	Name of school director or principal (interviewee) **Nombre del director(a) de la escuela (persona entrevistada)**	....
5	Name of interviewer **Entrevistador**	....
6	**A** – Does the school have a deworming program? **¿Tiene la escuela un programa de desparasitación?**	☐ Yes **Sí** (1) ☐ No **No** (0) ☐ *Don't know* ***No sabe*** (99)
	B – If yes, in what year did the deworming program begin? **En caso de sí, ¿en qué año comenzó el programa?** ☐ *Not applicable (NA)* ***No aplica (NA)***	Year program began (yyyy): **Año que comenzó (aaaa)** .... ☐ *Don't know* ***No sabe*** (99)
	C - If yes, when was deworming last given to all children (in months and/or days)? **En caso de sí, ¿cuándo fue la última vez que se desparasitaron los niños (en meses y/o días)?** ☐ *Not applicable (NA)* ***No aplica (NA)***	________months, **meses** ________days **días** ☐ *Don't know* ***No sabe*** (99)
	D- If yes, how often is deworming given? **En caso de sí, ¿con qué frecuencia se realiza la desparasitación?** ☐ *Not applicable (NA)* ***No aplica (NA)***	______ time(s) every **vez/ veces cada** ____ year(s) **año(s)** ☐ *Don't know* ***No sabe*** (99)
7	What are the classroom floors made of? **¿De qué material está hecho el piso de las aulas de clase?**	☐ Tiles **Ladrillo** (0) ☐ Earth **Tierra** (2) ☐ *Don't know* ***No sabe*** (99) ☐ Cement **Cemento** (1) ☐ Other: **Otro** ____________________ (3)

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SCHOOL COMPOSITION
COMPOSICIÓN DE LA ESCUELA

8	How many education levels are there in the school? **¿Cuántos niveles de educación hay en la escuela?**	☐ Only primary **Sólo primaria** (1) ☐ Pre-school and primary **Pre-escolar y primaria** (2) ☐ Primary and secondary **Primaria y secundaria** (3) ☐ *Don't know* ***No sabe*** (99)	
9	**A-** How many grade 1 classes are there in the school? **¿Cuántas secciones de 1° grado hay en la escuela?**	______ Grade 1 classes **Secciones 1° grado**	☐ *Don't know* ***No sabe*** (99)
	B- How many grade 1 students (boys and girls) are there in the school? **¿Cuántos estudiantes (niños y niñas) de 1° grado hay en la escuela?**	______ Grade 1 boys; **Niños** ______ Grade 1 girls **Niñas**	☐ *Don't know* ***No sabe*** (99)
10	**A-** How many grade 2 classes are there in the school? **¿Cuántas secciones de 2° grado hay en la escuela?**	______ Grade 2 classes **Secciones 2° grado**	☐ *Don't know* ***No sabe*** (99)
	B- How many grade 2 students (boys and girls) are there in the school? **¿Cuántos estudiantes (niños y niñas) de 2° grado hay en la escuela?**	______ Grade 2 boys; **Niños** ______ Grade 2 girls **Niñas**	☐ *Don't know* ***No sabe*** (99)
11	**A-** How many grade 3 classes are there in the school? **¿Cuántas secciones de 3° grado hay en la escuela?**	______ Grade 3 classes **Secciones 3° grado**	☐ *Don't know* ***No sabe*** (99)
	B- How many grade 3 students (boys and girls) are there in the school? **¿Cuántos estudiantes (niños y niñas) de 3° grado hay en la escuela?**	______ Grade 3 boys; **Niños** ______ Grade 3 girls **Niñas**	☐ *Don't know* ***No sabe*** (99)
12	**A-** How many grade 4 classes are there in the school? **¿Cuántas secciones de 4° grado hay en la escuela?**	______ Grade 4 classes **Secciones 4° grado**	☐ *Don't know* ***No sabe*** (99)
	B- How many grade 4 students (boys and girls) are there in the school? **¿Cuántos estudiantes (niños y niñas) de 4° grado hay en la escuela?**	______ Grade 4 boys; **Niños** ______ Grade 4 girls **Niñas**	☐ *Don't know* ***No sabe*** (99)
13	**A-** How many grade 5 classes are there in the school? **¿Cuántas secciones de 5° grado hay en la escuela?**	______ Grade 5 classes **Secciones 5° grado**	☐ *Don't know* ***No sabe*** (99)
	B- How many grade 5 students (boys and girls) are there in the school? **¿Cuántos estudiantes (niños y niñas) de 5° grado hay en la escuela?**	______ Grade 5 boys; **Niños** ______ Grade 5 girls **Niñas**	☐ *Don't know* ***No sabe*** (99)

14	**A-** How many grade 6 classes are there in the school? **¿Cuántas secciones de 6º grado hay en la escuela?**	---------------------- Grade 6 classes **Secciones 6º grado**		☐ *Don't know* ***No sabe*** (99)
	B- How many grade 6 students (boys and girls) are there in the school? **¿Cuántos estudiantes (niños y niñas) de 6º grado hay en la escuela?**	---------- Grade 6 boys; **Niños**	---------- Grade 6 girls **Niñas**	☐ *Don't know* ***No sabe*** (99)

QUESTIONNAIRE – WATER
AGUA

15	**A-** Is there water in the school? **¿Hay agua en la escuela?**	☐ Yes **Sí** (1) ☐ No **No** (0) ☐ *Don't know* ***No sabe*** (99)
	B- If yes (to A), do the students drink this water? **En caso de sí, ¿Beben de esta agua los estudiantes?** ☐ *Not applicable (NA)* ***No aplica (NA)***	☐ Yes **Sí** (1) ☐ No **No** (0) ☐ *Don't know* ***No sabe*** (99)
	C- If yes (to A), where does the school's water come from? **En caso de sí, ¿de dónde viene el agua de la escuela?** ☐ *Not applicable (NA)* ***No aplica (NA)***	☐ Well with pump **Pozo con bomba** (0) ☐ Well without pump **Pozo sin bomba** (1) ☐ Water truck **Carro cisterna** (2) ☐ Tank **Tanque** (3) ☐ Potable (tap) water **Acueducto (llave)** (4) ☐ Rain water **Lluvia** (5) ☐ Other:______________ **Otro** (6) ☐ *Don't know* ***No sabe*** (99)
	D- If yes (to A), does the school use chlorine to treat the water? **En caso de sí, ¿tratan el agua con cloro en la escuela?** ☐ *Not applicable (NA)* ***No aplica (NA)***	☐ Yes **Sí** (1) ☐ No **No** (0) ☐ *Don't know* (99)
16	**A-** Does the school have water available all day? **¿Dispone de agua la escuela durante todo el día?**	☐ Yes **Sí** (1) ☐ No **No** (0) ☐ *Don't know* ***No sabe*** (99)
	B- If no, when is water available? **En caso de no, ¿en qué momento disponen de agua?** ☐ *Not applicable (NA)* ***No aplica (NA)***	*If no, water is available:* **En caso de no, el agua está disponible sólo:** --

QUESTIONNAIRE – SANITATION
HIGIENE

17	**A-** Are there toilet facilities for the students of the school? **¿Hay instalaciones sanitarias para los estudiantes?**	☐ Yes **Sí** (1) ☐ No **No** (0)	☐ *Don't know* ***No sabe*** (99)
	B- If yes (to A), are there separate toilet facilities for boys and girls? **En caso de sí, ¿están las instalaciones sanitarias separadas por género (niña/ niño)?** ☐ *Not applicable (NA)* ***No aplica (NA)***	☐ Yes **Sí** (1) ☐ No **No** (0)	☐ *Don't know* ***No sabe*** (99)
	C- If yes (to A), are the toilet facilities in a separate building from the school? **En caso de sí, ¿están las instalaciones sanitarias separadas de la escuela?** ☐ *Not applicable (NA)* ***No aplica (NA)***	☐ Yes **Sí** (1) ☐ No **No** (0)	☐ *Don't know* ***No sabe*** (99)
18	**A-** Are there functioning, flushable toilets for the students in the school? **¿Hay servicios sanitarios para los estudiantes?**	☐ Yes **Sí** (1) ☐ No **No** (0)	☐ *Don't know* ***No sabe*** (99)
	B- If yes, what type of toilet? **En caso de sí, ¿qué tipo de inodoro?** ☐ *Not applicable (NA)* ***No aplica (NA)***	☐ Structure above ground **Estructura sobre el terreno** (1) ☐ Hole **Hoyo** (2)	☐ Other:____________ **Otro** (3) ☐ *Don't know* ***No sabe*** (99)
19	Is there a functioning latrine for the students of the school? **¿Hay letrinas funcionales para los estudiantes?**	☐ Yes **Sí** (1) ☐ No **No** (0)	☐ *Don't know* ***No sabe*** (99)
20	**A-** Are there sinks for the students to wash their hands in the school? **¿Los estudiantes tienen lavamanos disponibles?**	☐ Yes **Sí** (1) ☐ No **No** (0)	☐ *Don't know* ***No sabe*** (99)
	B- If yes, do the sinks have potable (tap) water? **En caso de sí, ¿estos lavamanos cuentan con agua potable?** ☐ *Not applicable (NA)* ***No aplica (NA)***	☐ Yes **Sí** (1) ☐ No **No** (0)	☐ *Don't know* ***No sabe*** (99)

21	**A-** Is there soap available in the school for students to wash their hands? **¿Los estudiantes tienen jabón disponible para lavarse las manos?**	☐ Yes **Sí** (1) ☐ No **No** (0) ☐ *Don't know* ***No sabe*** (99)
	B- If no, why is it not available? **En caso de no, ¿por qué no tienen jabón disponible?** ☐ *Not applicable (NA)* ***No aplica (NA)***	*If no, **reason:*** **Razón:** ---------- ----------

		Yes **Sí** (1)	No **No** (0)	*NA* ***NA***	In General **<u>En General</u>**
22	How are the conditions of the toilet or latrine (if facilities are not separate for boys/girls)? **En caso de que las instalaciones sanitarias no estén separadas por género, ¿cómo son las condiciones del inodoro o letrina?** ☐ *Not applicable (NA)* ***No aplica (NA)***	☐	☐	☐	-The toilet or latrine is functional - **Es funcional**
		☐	☐	☐	-The toilet or latrine is clean - **Está limpio**
		☐	☐	☐	-There is toilet paper available - **Tiene papel higiénico**
		☐	☐	☐	-There is a clean cloth or paper towel to dry the hands - **Tiene toalla limpia o papel toalla**
		☐	☐	☐	-The garbage is covered or emptied often - **La basura está tapada o se bota frecuentemente**
		☐	☐	☐	-There is no dirty toilet paper on the floor - **No hay papel higiénico sucio en el piso**
		☐	☐	☐	-Has a good smell - **Tiene buen olor (no huele mal)**
		☐	☐	☐	-The doors are on the hinges - **Las puertas tienen visagras**
		☐	☐	☐	-The floor is frequently cleaned - **El piso se limpia frecuentemente**

		Yes **Sí** (1)	No **No** (0)	*NA* ***NA***	Boys **<u>Niños</u>**
23	How are the conditions of the boys' toilet or latrine? **¿Cómo son las condiciones del inodoro o letrina de los niños?** ☐ *Not applicable (NA)* ***No aplica (NA)***	☐	☐	☐	-The toilet or latrine is functional - **Es funcional**
		☐	☐	☐	-The toilet or latrine is clean - **Está limpio**
		☐	☐	☐	-There is toilet paper available - **Tiene papel higiénico**
		☐	☐	☐	-There is a clean cloth or paper towel to dry the hands - **Tiene toalla limpia o papel toalla**
		☐	☐	☐	-The garbage is covered or emptied often - **La basura está tapada o se bota frecuentemente**
		☐	☐	☐	-There is no dirty toilet paper on the floor - **No hay papel higiénico sucio en el piso**
		☐	☐	☐	-Has a good smell - **Tiene buen olor (no huele mal)**
		☐	☐	☐	-The doors are on the hinges - **Las puertas tienen visagras**
		☐	☐	☐	-The floor is frequently cleaned - **El piso se limpia frecuentemente**
		Yes **Sí** (1)	No **No** (0)	*NA* ***NA***	Girls **<u>Niñas</u>**
24	How are the conditions of the girls' toilet or latrine? **¿Cómo son las condiciones del inodoro o letrina de las niñas?** ☐ *Not applicable (NA)* ***No aplica (NA)***	☐	☐	☐	-The toilet or latrine is functional - **Es funcional**
		☐	☐	☐	-The toilet or latrine is clean - **Está limpio**
		☐	☐	☐	-There is toilet paper available - **Tiene papel higiénico**
		☐	☐	☐	-There is a clean cloth or paper towel to dry the hands - **Tiene toalla limpia o papel toalla**
		☐	☐	☐	-The garbage is covered or emptied often - **La basura está tapada o se bota frecuentemente**
		☐	☐	☐	-There is no dirty toilet paper on the floor - **No hay papel higiénico sucio en el piso**
		☐	☐	☐	-Has a good smell - **Tiene buen olor (no huele mal)**
		☐	☐	☐	-The doors are on the hinges - **Las puertas tienen visagras**
		☐	☐	☐	-The floor is frequently cleaned - **El piso se limpia frecuentemente**

Comments or observations:
Comentarios u Observaciones:

THANK YOU FOR PARTICIPATING! ¡GRACIAS POR SU PARTICIPACIÓN!

APPENDIX K – Interview guideline (school questionnaire)

INTERVIEW GUIDE – SCHOOL QUESTIONNAIRE

FOR SELECTED QUESTIONS

Project Title: Gender and parasitic diseases: Integrating gender analysis in epidemiologic research on parasitic diseases to optimize the impact of prevention and control measures

This questionnaire is to be administered to school principal/director

4	Name of person who is answering questions (interviewee).
6C	Indicate how many months AND/OR days ago the LAST deworming was given to all students.
6D	(eg. *1* time every *2* years)
8	This question will likely need to be adapted depending on local school system.
9-14	Again, this may need to be adapted (add or remove grades) depending on school system and how many grades can exist in a primary school.
15B-D	Ask questions 15 B,C,D if the answer to 15A is "yes"; NA to 15 B,C,D if answer to 15A is "no".
17 B-C	Ask both questions if the answer to 17A is "yes"; NA to B-C if answer to A is "no".
22 - 24	Q 22: if the toilet/latrine facilities are NOT SEPARATE for boys and girls. Q 23/24: if the toilet/latrine facilities ARE SEPARATE for boys and girls . (23 = boys' facilities; 24 = girls' facilities)
Comments	Any relevant comment/observations about the school that could have an effect on STHs (eg. Cleaning, deworming, additional programs, etc).

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